Effect of Catalyst-Assisted Hydrothermal/Organosolv Process for Fractionation of Parawood

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Abstract. Fractionation of lignocellulosic components is a pre-requisite in biorefineries in order to separate the main compositions of raw material for further maximum utilization to biofuels, chemicals, and materials. Generally, a single step process is often insufficient to assure full availability of polymeric sugars from raw material. Therefore, the purpose of this study is to investigate two-step fractionation comprising hydrothermal and organosolv processes for the efficient separation of lignocellulosic biomass with a promising recovered yield in terms of quality and quantity. In this study, the effects of acids and bases concentration were studied in the two-step fractionation process, since they play different important roles in separation process. It was observed that the optimal condition of hydrothermal process was performed at 180°C for 60 min in the presence of 3.3% (w/w) of H₂SO₄ followed by organosolv process at 200°C for 60 min with 5% (w/w) of NaOH, resulting in the efficiency of hemicellulose and lignin removal of 87.96% and 46.39%, respectively. In addition, the high recovery of hemicellulose as pentose sugars were observed. The remaining cellulose was obtained with 80.22%. These results demonstrated the successive hydrothermal and organosolv process for fractionation of Parawood.

1. Introduction
Currently, energy needs are the driving force for exploring new energy resources. Biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power, materials and chemicals from agricultural residues. A biorefinery takes advantage on valorization of various components from biomass feedstock for value-added products. Agricultural biomass is not only considered as an interesting raw material for production of alternative energy but also a carbon-neutral raw material, therefore it does not cause global warming.

Lignocellulosic biomass is one of the most abundant carbon sources, and the most often used raw material for bioenergy production, since it is highly available and renewable. The use of lignocellulosic biomass, which includes energy crops, agro-residues, and forest waste from industry, have gained worldwide attention in recent years, as it is cheap and available [1]. However, Lignocellulosic materials have a complex structure consisting of cellulose, hemicellulose, and lignin, resulting in a high
recalcitrant for degradation and resist on enzymatic digestion [2][3]. An efficient fractionation approach is thus required for separation of these biopolymers for subsequent valorization to different products.

Parawood is a light-colored medium-density tropical hardwood obtained from the Para rubber tree (Hevea brasiliensis). Parawood is one of the most important economic trees in Thailand due to its availability as one of the main plantation crops in Asia [4]. The constituent biopolymers can be separated for further conversion to fuels, chemicals, and materials in biorefineries.

Fractionation of lignocellulosic components represent an initial step for value-added utilization of the individual biopolymers in integrated biorefineries. The fractionation process entails disruption of lignocellulose biopolymeric matrix and subsequent separation of its components into isolated fractions according to selectivity of the reaction media and process conditions [5]. Several techniques have been developed for biomass fractionation including various chemical (acid, alkaline, and oxidation) and thermochemical methods (steam explosion, autohydrolysis, and organosolv) or their combinations in multi-stage processes [6].

A two-step process comprising hydrothermal and organosolv processes are used for fractionation of lignocellulosic components from Parawood. Hydrothermal process using liquid hot water (LHW) is a physico-chemical pretreatment method that are used for specific separation of hemicellulose content and has been widely applied to enhance cellulose digestibility of various types of agricultural residues [7]. Hydrothermal process has several advantages over other leading pretreatment methods (e.g. diluted acid, alkali, and steam explosion) in that it has no chemical requirement and produces less inhibitory byproducts such as HMF and furfural [8]. The superheated liquid water auto-ionizes into hydronium ions, which act as a promoter for cleavage of ester bonds of acetyl side chains of hemicelluloses resulting in the formation of acetic acid [9]. The in situ generated acid then autocatalyzes hydrolysis of the hemicellulose and alteration of lignin structure, leading to increased enzyme accessibility to the cellulose fibers. Apart from Hydrothermal process, several homogeneous acids have been used as external catalysts in hydrothermal process of biomass. Dilute sulfuric are normally used to enhance hemicellulose solubilization from various agricultural residues [10-12]. However, the lignin that cannot be removed via hydrothermal process is partly rearranged on the surface of the lignocellulosic biomass exhibiting an inhibitory effect on downstream enzymatic hydrolysis [13].

Organosolv process has attracted a research interest, as it proposes an effective method to remove lignin with the use of organic solvents. The benefits of organosolv process is the isolation of high-quality lignin and high-purity lignin-free cellulose [14,15]. The lignin recovered is sulfur free, while the organic solvents used (ethanol) can easily be recovered which is a significant advantage for small scale biorefinery plants [16]. The addition of an organic solvent allows for better mass transfer and the dissolution of lignin [17], reducing its recondensation on the external surface area of the pulp [18]. Typically, Organosolv process are catalytically assisted with bases, such as NaOH. Sodium hydroxide can enhance lignin removal and lead to biomass swelling by solvation and saponification with minimal hydrolysis of the polysaccharide fractions [10-12].

However, using single step process, it is often difficult to achieve the appropriate biomass decomposition, thus the above-mentioned methods are combined to increase efficiency the separation of lignocellulosic biomass with a promising recovered yield in terms of quality and quantity [19,20]. For these reasons, there is significant research interest in investigating the best fractionation method for lignocellulosic materials. Sequential hydrothermal process for solubilization of hemicellulose and organosolv process for the removal of lignin and the production of high purity pulps have been published [15,21,22]. These studies investigated the effect of the different pretreatment techniques on the physical and chemical properties of the pulps, together with the saccharification effect of the residual solid.

In order to explore the use of external catalyst concentration on fractionation process, this study reports the use of H2SO4 as an acid catalyst for hydrothermal process and the use of NaOH as a base catalyst for organosolv process of Parawood. The effect of the catalyst concentration for improving hemicellulose/lignin solubilization was tested by measuring sugar recovery and yield in the liquid fraction, saccharified solid faction and lignin yield. An efficient two-step process will be optimized on
reaction conditions based on process parameters to achieve high fractionation efficiency with a high quality of derived products.

2. Materials and methods

2.1. Materials
Parawood was provided from Asia Biomass Co., Ltd., Thailand. The biomass was physically reduced using a cutting mill and sieved to the particle size of 0.5-1 mm, and then dried at 70°C for 24 hours. The main composition of Parawood contained 32.06 wt% cellulose, 23.76 wt% hemicellulose and 31.28 wt% lignin with minor contents of 5.10 wt% ash and 7.80 wt% extractive, according to the standard NREL method [23].

2.2. Methods

2.2.1. Hydrothermal process. The process was performed in a 600 ml stainless-steel high-pressure reactor (Parr Instrument Company). Biomass loading was 10% with a working volume of 100 ml. The first stage was maintained at a constant temperature of 180°C for 60 min in the absence and presence of sulfuric acid (H₂SO₄) by varying concentrations (based on %w/w of biomass loading) and the initial pressure was adjusted at 20 bars by nitrogen. After LHW process, the solid fraction was separated by filtration by filter paper using a Buchner funnel and washing with deionized water until pH was neutral. The obtained solid was then dried at 70°C for further fractionation in the second stage. Chemical compositions of the pretreated solids were analyzed using the standard NREL method. The liquid fraction was collected for analysis of sugar and inhibitory by-products by High Performance Liquid Chromatography (HPLC).

2.2.2. Organosolv process. The remaining solid fraction from hydrothermal process (180°C, 3.3% H₂SO₄, 60min) was used as material in organosolv process. The organic solvent was an aqueous solution of ethanol (95% v/v). The parameters of the second stage were maintained at 200°C for 60 min. The process was performed at different sodium hydroxide (NaOH) concentration (based on %w/w of biomass loading). After organosolv process, the solid fraction was separated from the liquid fraction by filtration. In liquid phase, the isolated lignin was separated by precipitation technique. All obtained fractions were collected for further analysis.

Figure 1. Process flow chart of the two-step fractionation.
2.2.3. Lignin precipitation. According to the practical procedure for precipitation of lignin, DI water was added with 3 time based on the initial volume of liquid fraction, and then the pH was adjusted equal to 2 by sulfuric acid. The supernatant was collected at room temperature for 24 h. After precipitation was completed, lignin was separated by filtration by filter paper using a Buchner funnel and dried for further utilization.

2.2.4. Analytical methods. The composition of raw material and pretreated biomass, including cellulose, hemicellulose and lignin was determined according to National Renewable Energy Laboratory (NREL) as standard protocols. The profiles of sugars and contaminated products (e.g. lactic acid, formic acid, acetic acid, levulinic acid, HMF and furfural) in the liquid fractions were analyzed by high performance liquid chromatograph using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operating at 65°C with 5 mM H₂SO₄ as the mobile phase with the flow rate of 0.5 mL/min. The percentage of biomass composition (cellulose, hemicellulose and lignin) was determined based on the remaining contents in the remaining solid compared to their respective contents in the raw material. The calculations of remaining cellulose, hemicellulose and lignin removal were described according to the following equations, respectively.

\[
\% \text{ Remaining cellulose} = \frac{C_F}{C_I} \times 100 \quad (1)
\]

\[
\% \text{ Hemicellulose removal} = \frac{H_I - H_F}{H_I} \times 100 \quad (2)
\]

\[
\% \text{ Lignin removal} = \frac{L_I - L_F}{L_I} \times 100 \quad (3)
\]

Where, Remaining cellulose is the percentage of remaining cellulose after fractionation process compared to initial cellulose content (%); \( C_F \) is the total amount of remaining cellulose content in solid fraction after the process (g); \( C_I \) is the total amount of cellulose in the raw material (g) and hemicellulose removal efficiency is the percentage of hemicellulose removal after fractionation process compared to initial hemicellulose content (%); \( H_I \) is the total amount of hemicellulose in the raw material (g); \( H_F \) is the total amount of remaining hemicellulose content in solid fraction after the process (g) and lignin removal efficiency is the percentage of lignin removal after fractionation process compared to initial lignin content (%); \( L_I \) is the total amount of lignin in the raw material (g); \( L_F \) is the total amount of remaining lignin content in solid fraction after the process (g).

The C₅ sugars recovery and C₅ sugars and lignin yield were calculated as the percentage of the obtained product based on content in the raw material (dry weight basis).

\[
\text{Product recovery} = \frac{\text{Grams of the product obtained}}{\text{Grams of the product in raw material} \times \text{Percentage of the product removal}} \times 100 \quad (4)
\]

\[
\text{Product yield} = \frac{\text{Grams of the product obtained}}{\text{Grams of the product in raw material}} \times 100 \quad (5)
\]

3. Results and discussion

3.1. Hydrothermal process
The effect of acid catalyst concentration in hydrothermal process for fractionation of parawood was studied by varying H₂SO₄ concentration (without acid catalyst, 0.6%w/w, 3.3%w/w, and 12.5%w/w). The operating condition was kept constant at 180°C for 60 min. The compositional changes in solids fraction after hydrothermal process are summarized in Table 1. Acidic condition plays an important role in separation of hemicellulose content. It was found that standard hydrothermal process showed particularly removal of hemicellulose fraction. According to Figure 2, the presence of H₂SO₄-catalyzed led to higher removal of hemicellulose than non-catalytic process. The highest hemicellulose removal
was obtained at 12.5% H$_2$SO$_4$ with 94.95%. This indicates the increasing of acid concentration led to an increasing trend on solubilization of hemicellulose. For further purpose of this step, it was proven that the hydrothermal process demonstrated a less effect on the fraction of lignin and cellulose. However, the severe condition in fractionation could affect the degradation of derived sugars as the results of products in liquid fraction.

**Table 1.** The composition of remaining solid after hydrothermal process at 180°C for 60 min under different acid concentration

| Run | Process condition          | H$_2$SO$_4$ concentration (%) | Cellulose (%)$^a$ | Hemicellulose (%)$^a$ | Lignin (%)$^a$ | Ash (%)$^a$ |
|-----|----------------------------|-------------------------------|-------------------|-----------------------|---------------|-------------|
| 1   | without acid catalyst      | 31.28                         | 6.19              | 28.02                 | 1.00          |
| 2   | 0.6                        | 31.46                         | 5.36              | 25.48                 | 1.93          |
| 3   | 3.3                        | 31.47                         | 3.45              | 25.27                 | 1.83          |
| 4   | 12.5                       | 29.23                         | 1.20              | 23.33                 | 1.50          |

$^a$ % Dry weight basis

**Figure 2.** The efficiency of hydrothermal process on the response of hemicellulose removal, lignin removal and remaining cellulose under different acid concentration at 180°C for 60 min.

Tables 2 and 3 show the product profiles in liquid fraction after hydrothermal process at 180°C for 60 min under acidic condition. The product was analyzed in form of derived sugars and contaminated products. Hydrothermal in the presence of acid catalyst led to extensive solubilization and hydrolysis of the hemicelluloses into sugars and by-products. Increasing of H$_2$SO$_4$ concentration led to increase C$_5$ sugars concentration. This result demonstrated that C$_5$ recovery was substantial declined due to the degradation of sugars into by-products. In contrast, addition of the acid concentration from 0.6% to 3.3% in hydrothermal process led to increased C$_5$ sugars yield compared to standard condition. Further increasing the acid concentration above 12.5% led to decreased C$_5$ yield due to the marked increased
formation of side-products. Generally, furfural is released from degradation of pentose sugars, while hydroxymethylfurfural (HMF) is generated from degradation of hexose sugars. Particularly, the high amount of HMF and furfural were found under the harsh condition. Thus, the suitable condition of hydrothermal process was at 180°C for 60 min with the presence of 3.3% w/w H₂SO₄, resulting in almost complete of hemicellulose removal with the highest yield of C₅ sugars.

Table 2. The sugar profiles in liquid fraction after hydrothermal process at 180°C for 60 min under different acid concentration

| Run | H₂SO₄ concentration (% w/w) | C₆ sugars (g/L) | C₅ sugars (g/L) | Total C₅ Sugar (g) | C₅ recovery (%) | C₅ yield (%) |
|-----|-----------------------------|----------------|----------------|-------------------|-----------------|-------------|
|     |                             | Glucose | Xylose | Arabinose | Glucose | Xylose | Arabinose | Glucose | Xylose | Arabinose |
| 1   | without acid catalyst       | 0.24    | 1.61   | 2.29      | 8.42  | 0.46 | 0.52      | 1.17    | 58.54 | 43.29     |
| 2   | 0.6                         | 0.62    | 1.09   | 3.93      | 7.36  | 0.55 | 0.00      | 1.18    | 56.59 | 43.83     |
| 3   | 3.3                         | 1.71    | 0.28   | 9.04      | 2.44  | 0.00 | 0.00      | 1.24    | 53.78 | 45.97     |
| 4   | 12.5                        | 2.05    | 0.16   | 9.23      | 0.20  | 1.18 | 0.00      | 1.06    | 41.39 | 39.30     |

Table 3. The contaminated compounds in liquid fraction after hydrothermal process at 180°C for 60 min under different acid concentration (con.)

| Run | H₂SO₄ concentration (% w/w) | Concentration (g/L) | Lactic acid | Formic acid | Acetic acid | Levulinic acid | HMF | FF |
|-----|-----------------------------|----------------------|------------|------------|------------|----------------|-----|----|
| 1   | without acid catalyst       | 1.13                 | 1.75       | 8.98       | 0.00       | 0.13           | 0.78|
| 2   | 0.6                         | 1.46                 | 1.54       | 7.24       | 1.33       | 0.26           | 0.98|
| 3   | 3.3                         | 3.28                 | 2.59       | 9.34       | 0.00       | 0.79           | 1.74|
| 4   | 12.5                        | 4.88                 | 3.01       | 12.28      | 0.01       | 1.11           | 2.19|

3.2. Organosolv process
The second step fractionation experiments of Parawood were continually conducted. The remaining solid fraction from hydrothermal process (180°C, for 60 min with 3.3% w/w H₂SO₄) was used as material in organosolv process. The process was conducted by varying NaOH concentration (without basic catalyst, 5% w/w, 10% w/w). The operating conditions were kept constant at 200°C for 60 min. The compositional changes in solids fraction after two step process are summarized in Table 4. Since basic condition plays an important role in separation of lignin fraction. It was found that organosolv process in the presence of base catalyst could efficiently remove lignin fraction. According to Figure 3, the presence of NaOH-catalyzed led to higher removal of lignin than non-catalytic process. The highest lignin removal was obtained at 10% NaOH with 56.91%, which corresponded to the highest lignin yield (23.23%). However, a high NaOH concentration (10%) showed marked decrease of cellulose fraction due to the degradation of cellulose into contaminated products. According to the purpose of fractionation process, the high recovered yield of three fractions (cellulose, hemicellulose, and lignin) are required. This indicates that the optimal condition would be considered at the middle point in order to obtain the high yield of all desired fraction. Thus, the suitable condition of organosolv process was at 200°C for 60 min with the presence of 5% w/w NaOH, resulted in 46.39% of lignin removal and 19.25% of lignin yield.
Table 4. The composition of remaining solid after sequential organosolv process at 200°C for 60 min under different base concentration

| Run | Process conditions | NaOH concentration (% w/w) | Cellulose (%)<sup>a</sup> | Hemicellulose (%)<sup>a</sup> | Lignin (%)<sup>a</sup> | Ash (%)<sup>b</sup> | Lignin precipitation (g)<sup>b</sup> | Lignin yield (%)<sup>b</sup> |
|-----|---------------------|----------------------------|---------------------------|-----------------------------|--------------------|----------------|-----------------------------|-----------------------------|
| 1   | without basic catalyst | 3.3 | 28.03 | 3.28 | 18.21 | 1.90 | 0.5271 | 16.85 |
| 2   | 5                   | 25.72 | 2.86 | 16.77 | 1.50 | 0.6020 | 19.25 |
| 3   | 10                  | 21.52 | 2.65 | 13.48 | 2.40 | 0.7265 | 23.23 |

<sup>a</sup> % Dry weight basis

<sup>b</sup> Dry weight after precipitation process

Figure 3. The efficiency of sequential organosolv process on the response of hemicellulose removal, lignin removal and remaining cellulose under different base concentration at 200°C for 60 min

4. Conclusion
An integrated system of two step fractionation was found to have a significant impact on specific separation of lignocellulosic biomass. The specific separation of both hemicellulose and lignin in two step process was optimized. The suitable condition was observed as follows; first step: the presence of 3.3% H<sub>2</sub>SO<sub>4</sub> at 180°C for 60 min, second step: the presence of 5% NaOH at 200°C for 60 min. The work demonstrated a sequential process for the efficient separation of Parawood in integrated biorefinery.

Acknowledgements
The author would like to thank Thailand Advanced Institute of Science and Technology and Tokyo Institute of Technology (TAIST-Tokyo Tech) for funding, Kasetsart University for suggestions. This work was supported by Integrative Biorefinery Laboratory (IBL), National Science and Technology Development Agency (NSTDA).
References

[1] Mood, S.H., Golfeshan, A.H., Tabatabaei, M., Jouzani, G.S., Najafi, G.H., Gholami, M., Ardjmand, M. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. Renew. Sust. Energy Rev. 2013, 27, 77–93.

[2] Liu H., Zhang Y.X., Hou T., Chen X., Gao C., Han L., Xiao W. Mechanical deconstruction of corn stover as an entry process to facilitate the microwave-assisted production of ethyl levulinate. Fuel Process. Technol. 2018, 174:53–60. doi: 10.1016/j.fuproc.2018.02.011.

[3] Jönsson L.J., Martin C. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. Bioresour. Technol. 2016, 199:103–112. doi: 10.1016/j.biortech.2015.10.009.

[4] MAF Mazlan, Y Uemura, N Osman, S Yusup, Fast pyrolysis of hardwood residues using a fixed bed drop-type pyrolyzer, Ene Conv Manag 98 (2015) 208–214.

[5] Huang HJ, Ramaswamy S, Tschirner UW, Ramaraob BV. A review of separation technologies in current and future biorefineries. Sep Purif Technol 2008, 62:1-21.

[6] Hu, F., Ragauskas, A., 2012. Pretreatment and lignocellulosic chemistry. Bioenerg. Res. 5, 1043–1066.

[7] A. Kruse, E. Dinjus, Hot compressed water as reaction medium and reactant properties and synthesis reactions, J. Supercrit. Fluids 39 (2007) 362–380

[8] T.N. Ang, G.C. Ngoh, A.S.M. Chua, Comparative study of various pretreatment reagents on rice husk and structural changes assessment of the optimized pretreated rice husk, Bioresour. Technol. 135 (2013) 116–119.

[9] C.C. Teo, S.N. Tan, J.W.H. Yong, C.S. Hew, E.S. Ong, Pressurized hot water extraction (PHWE), J. Chromatogr. A 1217 (2010) 2484–2494.

[10] B. Yang, C.E. Wyman, Pretreatment: the key to unlocking low cost cellulosic ethanol, Biofuels Bioprod. Biorefin. 2 (2007) 26–40.

[11] J. Xu, J.J. Cheng, Pretreatment of switchgrass for sugar production with the combination of sodium hydroxide and lime, Bioresour. Technol. 102 (2011) 3861–3868.

[12] X. Li, T.H. Kim, Low-liquid pretreatment of corn stover with aqueous ammonia, Bioresour. Technol. 102 (2011) 4779–4786

[13] Kristensen, J.B.; Thygesen, L.G.; Felby, C.; Jørgensen, H.; Elder, T. Cell wall structural changes in wheat straw pretreated for bioethanol production. Biotechnol. Biofuels 2008, 1, 5.

[14] Zhang, K.; Pei, Z.; Wang, D. Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: A review. Bioresour. Technol. 2016, 199, 21–33.

[15] Huijgen, W.J.J.; Smit, A.T.; de Wild, P.J.; den Uil, H. Fractionation of wheat straw by prehydrolysis, organosolv delignification and enzymatic hydrolysis for production of sugars and ethanol. Bioresour. Technol. 2012, 114, 389–398

[16] Vallejos, M.E.; Zambon, M.D.; Area, M.C.; da Silva Curvelo, A.A. Low liquid-solid ratio fractionation of sugarcane bagasse by hot water autohydrolysis and organosolv delignification. Ind. Crops Prod. 2015, 65, 349–353.

[17] Oliet, M.; García, J.; Rodríguez, F.; Gilarranz, M. Solvent effects in autocatalyzed alcohol–water pulping. Chem. Eng. J. 2002, 87, 157–162.

[18] Oliet, M.; Rodríguez, F.; Gilarranz, M.A. The effect of autocatalyzed ethanol pulping on lignin characteristics. J. Wood Chem. Technol. 2001, 21, 81–95.

[19] Chen, H.; Liu, J.; Chang, X.; Chen, D.; Xue, Y.; Liu, P.; Li, H.; Han, S. A review on the pretreatment of lignocellulose for high-value chemicals. Fuel Process. Technol. 2017, 160, 196–206.

[20] Solarte-Toro, J.C.; Romero-García, J.M.; Martínez-Patiño, J.C.; Ruiz-Ramos, E.; Castro-Galiano, E.; Cardona-Alzate, C.A. Acid pretreatment of lignocellulosic biomass for energy vectors production: A review focused on operational conditions and techno-economic assessment for bioethanol production. Renew. Sust. Energy Rev. 2019, 107, 587–601.
[21] Garrote, G.; Falqué, E.; Domínguez, H.; Parajó, J.C. Autohydrolysis of agricultural residues: Study of reaction byproducts. Bioresour. Technol. 2007, 98, 1951–1957.

[22] Amendola, D.; De Faveri, D.M.; Egües, I.; Serrano, L.; Labidi, J.; Spigno, G. Autohydrolysis and organosolv process for recovery of hemicelluloses, phenolic compounds and lignin from grape stalks. Bioresour. Technol. 2012, 107, 267–274.

[23] Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2004). Laboratory analytical procedure (LAP). Golden: National Renewable Energy Laboratory (NREL).