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APPENDICES

Appendix A Chemical Composition of Rice Straw

The concentration of cell wall components was measured by using the Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Digestible Lignin (ADL) procedures (Van Soest *et al.*, 1991). The chemical composition was analyzed by the following equations:

$$NDF = Cellulose + Hemicellulose + Lignin \quad (A1)$$

$$ADF = Cellulose + Lignin \quad (A2)$$

$$ADL = Lignin \quad (A3)$$

Table A1 The chemical composition of untreated rice straw

Composition	Percentage
Cellulose	39.49
Hemicellulose	18.64
Acid detergent lignin	4.32
Others	37.55

Note : Others include extractive, acid soluble lignin, protein and ash.

Appendix B Effect of Acid Type on Total Sugar Yield

According to the results of Trisinsub, the optimum conditions for ionic liquid [Emim][Ac] pretreated rice straw samples at 50 volume% was obtained. In this work, [Emim][Ac] pretreated rice straw was first employed to study the effect of acid strength on the hydrolysis of cellulose to reducing sugar at the same conditions. The combination of [Emim][Ac] and acid, (HCl and CH_3COOH) were investigated in the present study. Although in an ionic liquid with strong H-bonding basicity need to dissolve cellulose, it was reported that some acids added in ionic liquid could enhance the total reducing sugar yield. However, it depends on the type of ionic liquid and pretreatment conditions. Previously, the combining ionic liquid and HCl into a pretreatment of sugarcane bagasse could enhance enzymatic saccharification of sugarcane bagasse (Zhang *et al.*, 2012).

As shown in Figure B1, pretreatment with 1 volume% HCl-aqueous [Emim][Ac] solution provided equivalent sugar yield to aqueous [Emim][Ac] solution and the sugar yields became lower at higher hydrochloric acid (HCl) concentrations as a result of small ionic-size of chloride anion (Cl^-). It can suitably interact with $[Emim]^+$. On the other hand, at any acetic acid (CH_3COOH) concentration, CH_3COOH-aqueous [Emim][Ac] solution had higher total sugar concentration than aqueous [Emim][Ac] solution. But, the sugar yields gave the same tendency of using hydrochloric acid (HCl) as a catalyst; total sugar concentration decreased at higher acetic acid (CH_3COOH) concentrations. It is possibly because increasing acetate anion (CH_3COO^-) concentration by adding acetic acid (CH_3COOH) can support acetate anion (CH_3COO^-) in ionic liquid ([Emim][Ac]) to improve pretreatment efficiency (Zhang *et al.*, 2012). Moreover, the size of chloride anion (Cl^-) in hydrochloric acid (HCl) is smaller than of acetate anion (CH_3COO^-) and interact with $[Emim]^+$. In addition, it could be explained by H-bonding basicity. Generally, hydroxyl group of cellulose interacts with the anion of ionic liquid which serves as H-bonding acceptor. The ability of ionic liquids to act as H-bonding acceptors is measured by H-bonding basicity (β). H-bonding basicity of acetate anion (CH_3COO^-) is 1.09, which is greater than of chloride anion (Cl^-) (0.87) (Ha *et al.*, 2011). From these reasons, acetate anion (CH_3COO^-) can interact with hydrogen atom in hydroxyl group better than chloride anion (Cl^-). Therefore, adding acetic acid (CH_3COOH) as a catalyst increases total sugar concentration and it is more effective than hydrochloric acid (HCl).

Figure B2 illustrates the SEM images of untreated and pretreated rice straw. After pretreatment, it was found that surface was rough and high rough surface when adding acid. Especially, adding acetic acid (CH_3COOH) in aqueous-[Emim][Ac].

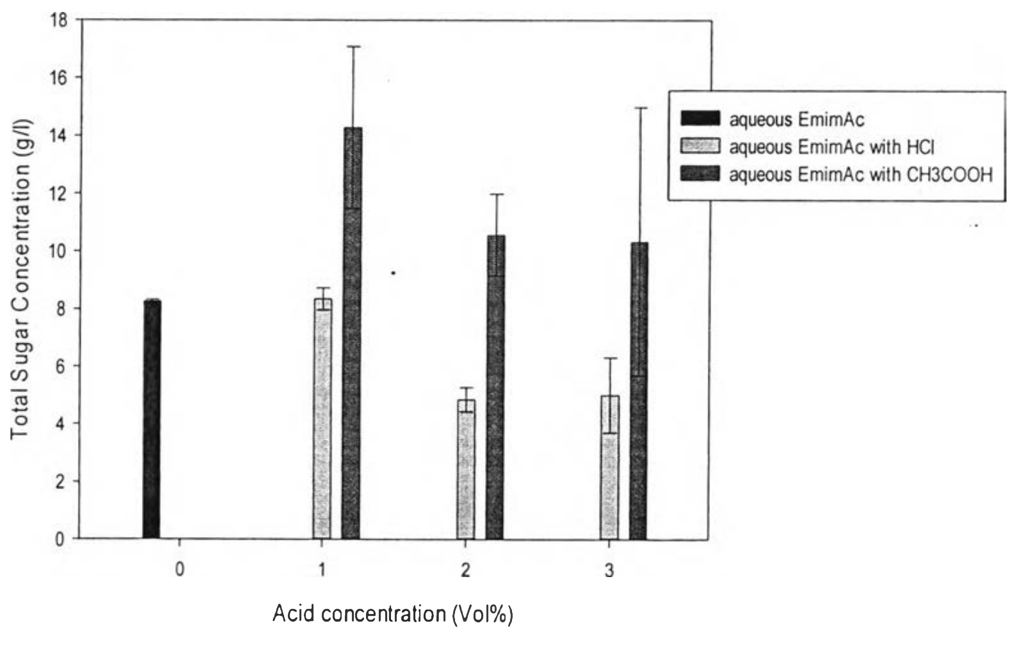

Figure B1 Comparison of total sugar concentration and acid type with different acid concentration by using rice straw as a raw material. Pretreatment conditions: 50 volume% [Emim][Ac] and 162 °C for 48 min.

The total sugar concentration of rice straw at microwave irradiation temperature and time of 147 °C, 76 min, respectively. 20 g/l and 1.287 volume% of biomass loading, and acetic acid was 14.01 g/l.

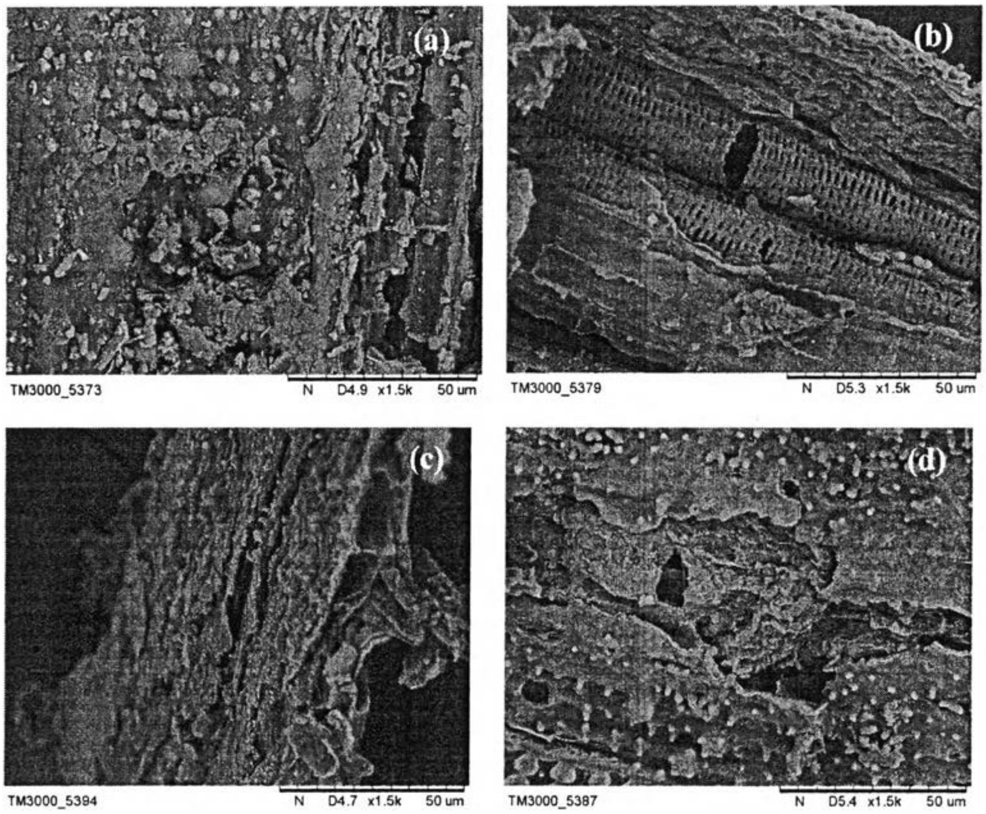

Figure B2 SEM images of untreated (a) and pretreated rice straw with aqueous-[Emim][Ac] (b), 1% CH3COOH aqueous-[Emim][Ac] (c), and 1% HCl aqueous-[Emim][Ac] at 50 volume% [Emim][Ac] and 162 °C for 48 min.

Appendix C Checking Reducing Sugar in Hydrolysate

The hydrolysate was collected and checked by HPLC. To check the sugar which might be occurred in hydrolysate. From HPLC, it did not show the peak of reducing sugar, but only showed acetic acid peak. Table B1 concluded that the amount of acetic acid in hydrolysate.

Table C1 The amount of acetic acid in hydrolysate

Sample	Acetic acid concentration (g/l)
Pakchong 1 (Leaf) Kan	44.76
Pakchong 1 (Stem) Kan	42.58
Jakkapat (Leaf)	34.14
Jakkapat (Stem)	37.53
Aqueous-[Emim][Ac]	120.31
1% HCl aqueous-[Emim][Ac]	142.41
3% HCl aqueous-[Emim][Ac]	74.20
2% CH_3COOH aqueous-[Emim][Ac]	106.77

For pretreated with hydrochloric acid, the detector could detect other peaks at retention time 6.6 min, as displayed in Figure C1. Might be hydrochloric acid peak.

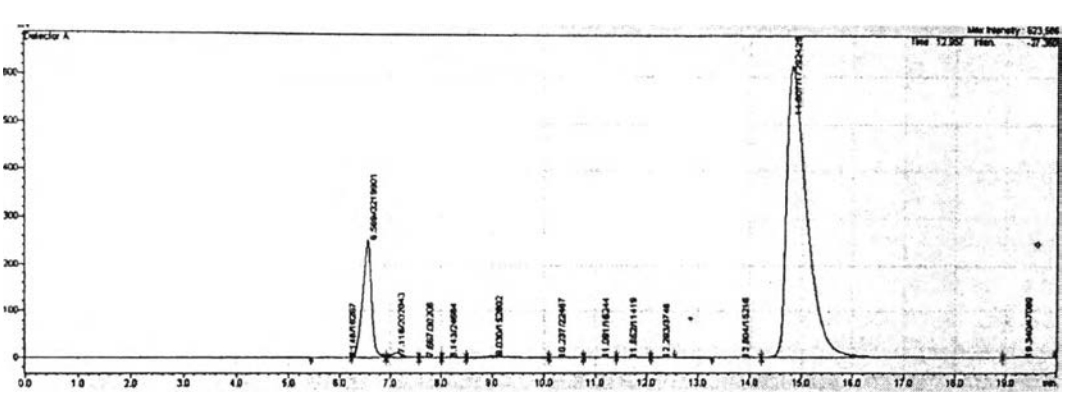

Figure C1 HPLC results of 3% HCl aqueous-[Emim][Ac].

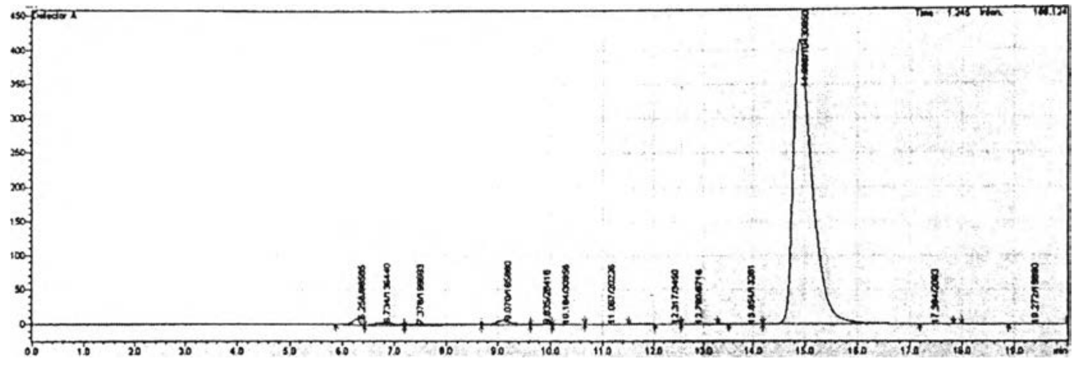

Figure C2 HPLC results of Pakchong 1 (Leaf) Kan.

Appendix D The Total Sugar Concentration at Any Acid Concentration

Table D1 depicted the value of total sugar concentration of pretreated with aqueous [Emim][Ac], CH_3COOH aqueous-[Emim][Ac], and HCl aqueous-[Emim][Ac] at 162 °C for 48 min. Furthermore, the dissolution mechanism of cellulose in CH_3COOH aqueous-[Emim][Ac] was shown in Figure D1.

Table D1 Comparison of total sugar concentration and acid type with different acid concentration by using Pakchong1 from Saraburi province as a raw material. Pretreatment conditions: 50 volume% [Emim][Ac] and 162 °C for 48 min

Acid concentration (volume%)	CH_3COOH	HCl
0	14.34 ± 1.38	
1	18.59 ± 0.07	14.85 ± 1.57
2	14.45 ± 0.37	13.00 ± 1.04
3	14.16 ± 0.66	11.01 ± 0.11
4	12.82 ± 0.23	13.11 ± 1.07

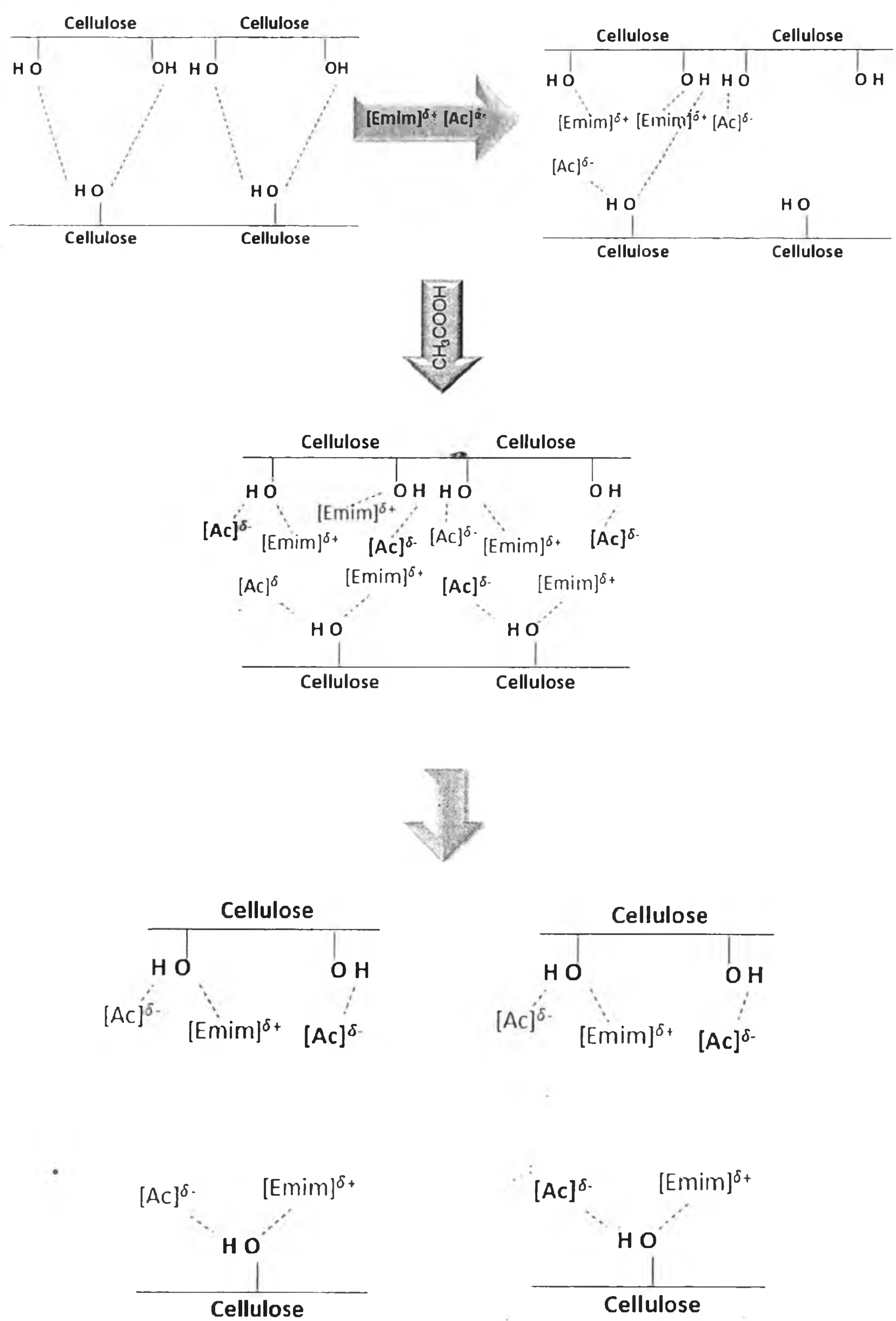

Figure D1 Dissolution mechanism of cellulose in CH_3COOH aqueous-[Emim][Ac] (Feng *et al.*, 2008; Zhang *et al.*, 2012).

Appendix E The Total Sugar Concentration of Mixed Napier Grass

Both leaf and stem of Napier grass were mixed in the ratio of 2:1 (leaf : stem). The total sugar concentration obtained is shown in Table E1. Theoretically, concentration should be higher than pretreated stem, but it was lower concentration. Maybe, because of the non-homogeneous of leaf and stem. They separated layer after mixed.

Table E1 The total sugar concentration of mixed Napier grass at 147 °C, 76 min, 20 g/l biomass loading, and 1.287 volume% acetic acid

Sample	Total sugar concentration (g/l)
Mix Pakchong 1 Kan	10.46 ± 0.30
Mix Pakchong 1 Sa	7.42 ± 0.05
Mix Jakkapat	8.98 ± 0.84

Appendix F Effect of Amount of Enzyme on Total Sugar Concentration

In this research used 66 μl/ g biomass of enzyme (52 FPU) which is the best condition for corn stover. Nevertheless, to confirm that this amount appropriate for Napier grass. Therefore, the amount of enzyme was investigated (66, 132, and 198 μl/ g biomass). Table F1 depicted that the high amount of enzyme was not significant.

Table F1 Total sugar concentration at different amount of enzyme on untreated Pakchong 1 from Saraburi province

The amount of enzyme (μl/g biomass)	Total sugar concentration (g/l)
66	2.42
132	2.07
198	2.06

Appendix G Composition of Pakchong 1 Leaf from Kanchanaburi Province by using NREL Method

Table G1 The composition of Napier grass was determined by the National Renewable Energy Laboratory (NREL)

Grass	Glucan	Xylan	Arabinan	Lignin	Ash	Extrac-tive
Untreated Pakchong1 (L)	33.756	19.403	6.139	18.498	0.908	36.443
Pretreated Pakchong1 (L)	41.831	20.291	5.944	11.684	0.913	31.811

Appendix H Manufacturing Overhead

Table H1 Solvents, chemicals, and materials cost

Item	Cost (Baht)
Solvents	
• 1-ethyl-3-methylimidazolium acetate ([Emim][Ac]), 1 kg	56,700
• Acetic acid, 99% purity 2.5 L	470
Chemicals	
• Cellulase from Trichoderma Reesei, 5 KU	7,200
• Sodium hydroxide, 1 kg	500
• Citric acid, 450 g	165
Materials	
• Pennisetum purpureum x P.glaucum Pakchong 1 (Pakchong 1 Napier grass), 1 kg	100
• Filter paper (Whatman No.1), 1 box (100 pcs.)	190

Pretreatment

- Ionic liquid ([Emim][Ac]) 5.5 g = 312 Baht
- Acetic acid 0.14 ml = 0.03 Baht
- Napier grass 0.22 g = 0.02 Baht
- Filter paper 2 pieces = 4 Baht
    - **Total** = 316.05 Baht

Hydrolysis (Using 20 mg pretreated Napier grass)

- Cellulase 13.2 μl = 0.013 Baht
- Citrate buffer 50 ml

- Citric acid 2.1 g = 0.77 Baht
- Sodium hydroxide 8 g = 4 Baht
- **Total** = 4.78 Baht

From hydrolysis result, total sugar concentration was 14.38 g/l. It could be calculated into g sugar/ g biomass unit, as shown below:

- Total sugar concentration 14.38 g/l
- Pretreated Napier grass 0.020 g
- Citrate buffer 0.001 l

$$\frac{14.38g}{1l} \times \frac{0.001l}{0.020g} = 0.72 gSugar / gBiomass$$

Therefore, 0.22 g Napier grass was produced 0.16 g sugar. It concluded that the manufacturing overhead of one batch was 320.83 Baht.

Appendix I Absorption and Desorption Isotherm

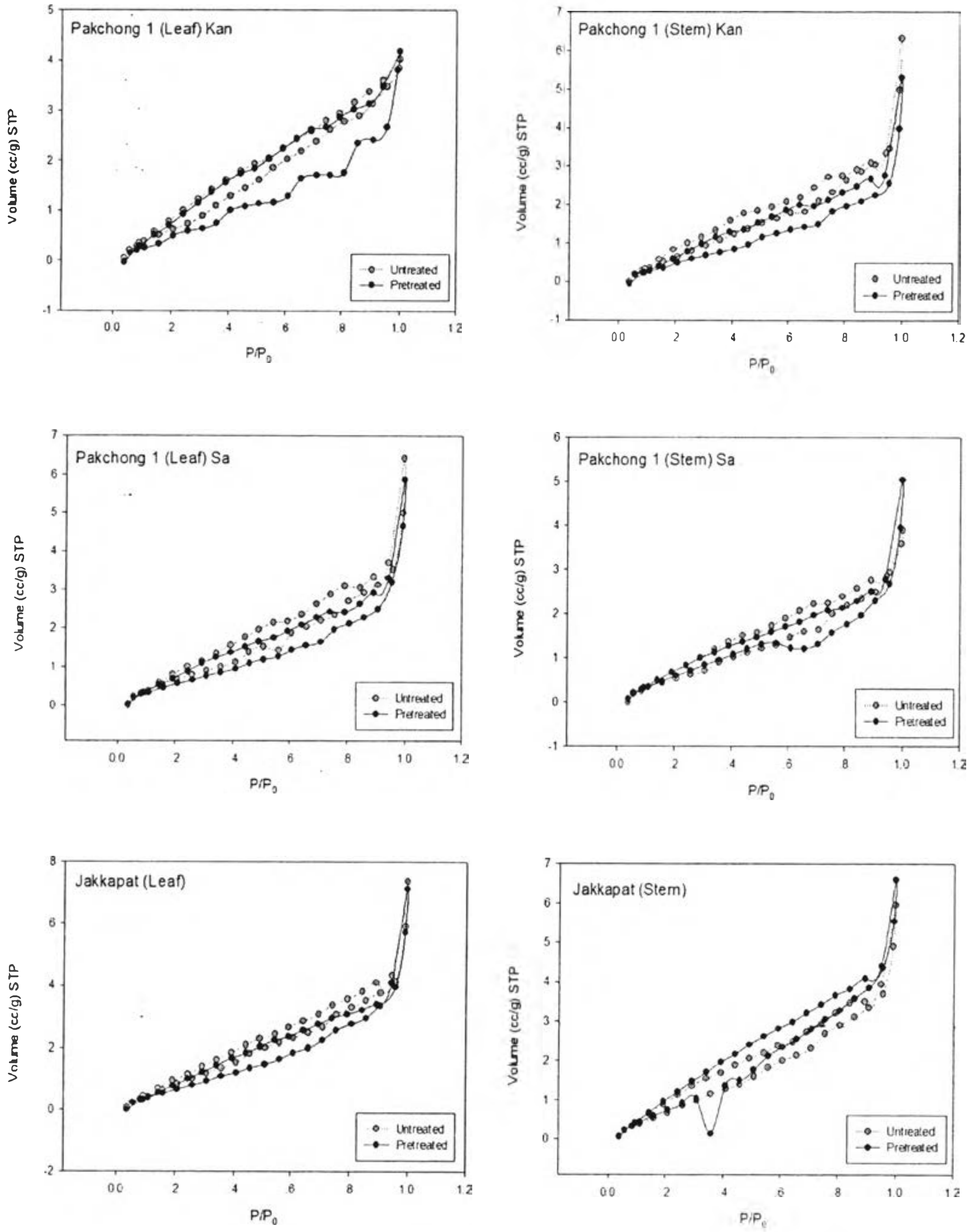

Figure I1 Absorption and desorption isotherm of untreated and pretreated Napier grasses. Reaction conditions: at 147 °C, 76 min, 20 g/l biomass loading, and 1.287 volume% acetic acid.

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Proceedings:

1. Wanapirom, R., Luengnaruemitchai, A., and Wongkasemjit, S. (2015, April 21) Lignocellulosic Biomass Pretreated with Ionic Liquid. Proceedings of The 6th Research Symposium on Petroleum, Petrochemicals, and Advanced Materials and The 21th PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.