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**APPENDICES**

**Appendix A**  
**Chemical Reagents**

All chemicals and their source in this study were given in Table A1.

Table A. List of some chemicals.

Lists	Sources
<b>Acids;</b>	
Acetic acid (CH <sub>3</sub> COOH; F.W. 60.05),	Labscan
2-furoic acid (C <sub>5</sub> H <sub>4</sub> O <sub>3</sub> ; F.W. 112.08), 98%	Aldrich
Phosphoric acid	
Sulfuric acid (H <sub>2</sub> SO <sub>4</sub> ; F.W. 98.08), 98%	Labscan
Tri-fluoro acetic acid (TFA, CF <sub>3</sub> COOH; F.W. 114.02), ≥98%	Aldrich
<b>Alkalis;</b>	
Potassium hydroxide (KOH; F.W. 56.11), 85%	Labscan
Sodium hydroxide (NaOH; F.W. 40), 97%	Labscan
<b>Solvents;</b>	
Acetone (CH <sub>3</sub> COCH <sub>3</sub> ; F.W. 58.08), ≥99%	Labscan
Acetonitrile (CH <sub>3</sub> CN; F.W. 41.05), HPLC grade ≥99%	Labscan
Ethanol (CH <sub>3</sub> CH <sub>2</sub> OH; F.W. 46.07), HPLC grade ≥99.5%	J.T.Baker
Furfural, GC grade 98%	Wako
Furfuryl alcohol	Fluka
Hydroxy-methyl-furfural (C <sub>6</sub> H <sub>6</sub> O <sub>3</sub> ; F.W. 126.11), GC grade ≥95%	Fluka
Water for HPLC grade	Labscan

Table A. List of some chemicals (cont.)

Lists	Sources
<b>Sugars;</b>	
L-(+)-Arabinose (C <sub>5</sub> H <sub>10</sub> O <sub>5</sub> ; F.W. 150.13), ≥99%	Himedia
D-(-)-Fructose (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> ; F.W. 180.16), ≥98%	Fluka
D-(+)-Galactose (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> ; F.W. 180.16), ≥99%	Sigma
D-(+)-Glucose (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> ; F.W. 180.16), ≥99%	Merck
D-(+)-Mannose (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> ; F.W. 180.16), ≥99%	Fluka
α-L-Rhamnose (C <sub>6</sub> H <sub>12</sub> O <sub>5</sub> ·H <sub>2</sub> O; F.W. 182.17), ≥99%	Fluka
D-(+)-Arabinose (C <sub>5</sub> H <sub>10</sub> O <sub>5</sub> ; F.W. 150.13), ≥99%	Sigma
<b>Salts:</b>	
Ammonium sulphate ((NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ; M.W. 132.14), ≥99.5%	Labscan
Copper sulphate (CuSO <sub>4</sub> ; M.W. 159.68), 98.5%	Ajax Finechem
Potassium dihydrogen orthophosphate (KH <sub>2</sub> PO <sub>4</sub> , M.W. 136.09), ≥99%	Ajax Finechem
Sodium chloride (NaCl; M.W. 58.43), ≥99.5%	Sigma-Aldrich
Sodium acetate (CH <sub>3</sub> COONa.3H <sub>2</sub> O; M.W. 136.08), ≥99%	Ajax Finechem
<b>Substances;</b>	
Bacteriopeptone	Himedia
Bovine serum albumin (BSA)	Sigma
Carboxy-methyl-cellulose (CMC)	Fluka
Cellulase from <i>Aspergillus niger</i>	Fluka
Malt extract	Himedia
Sodium chlorite (NaClO <sub>2</sub> )	Sigma-Aldrich
Vanillin	Sigma-Aldrich
Xylan	Sigma-Aldrich
Yeast extract	Labscan

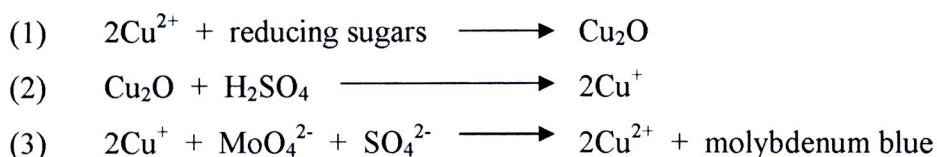
## Appendix B

### Sugar analysis and standard curve

#### 1. Colorimetric method

##### 1.1 Somogji-Nelson reagents

###### Reaction



##### 1.2 Chemical reagents

**1.2.1 Low-alkalinity reagent of Somogyi** was prepared by following below;

Reagent A: 12 g of Nak tartrate and 24 g anhydrous  $\text{Na}_2\text{CO}_3$  were dissolved in 250 ml boiled distilled water.

Reagent B: 4.0 g  $\text{Cu}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$  was dissolved in 50 ml distilled water.

Reagent C: 180 g anhydrous  $\text{Na}_2\text{SO}_4$  was dissolved in 500 ml distilled water and then heated for releasing air. After that, it was cooled.

“Adding reagent B in to reagent A together with mixing and then 16 g  $\text{NaHCO}_3$  was added slowly. After that, reagent C was added and finally 1000 ml was a final volume and adjusted by boiled distilled water. Keep this solution at  $37^\circ\text{C}$  for a week.”

**1.2.2 Arsenomolybdate reagent of Nelson** was prepared by following below;

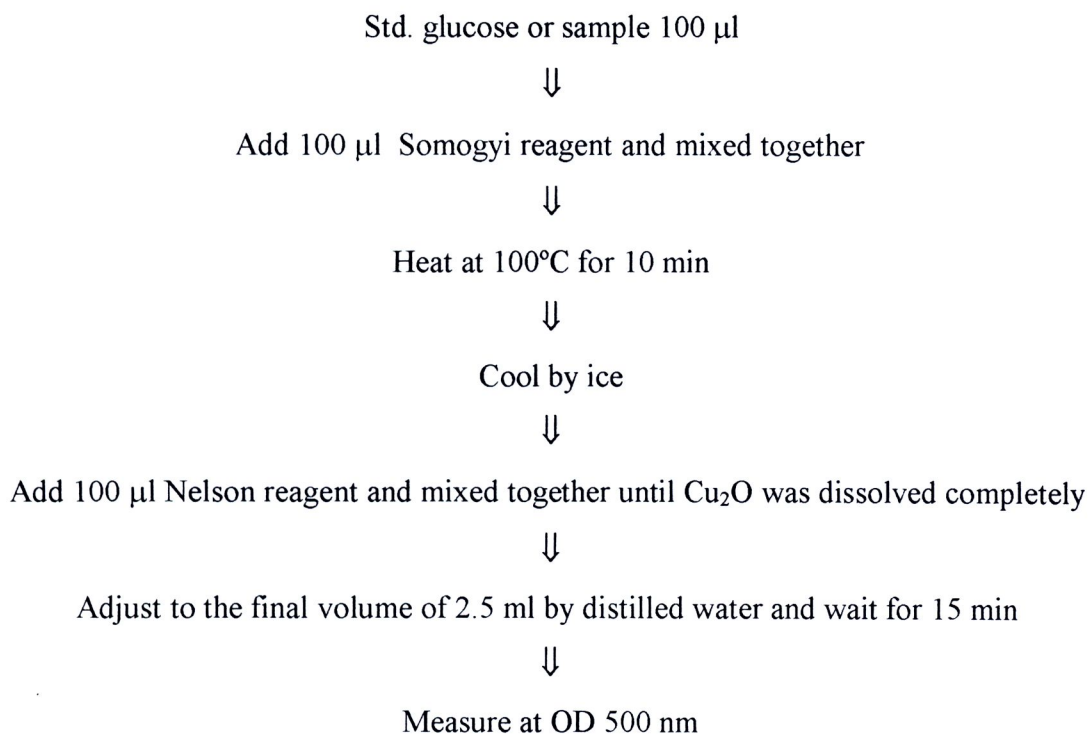
Reagent D: 25 g ammonium molybdate was dissolved by 450 ml distilled water and then added by 21 ml of 96% sulfuric acid.

Reagent E: 3.0 g  $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$  was dissolved by 25 ml distilled water.

“Reagent E was added to reagent D and adjusted to 1000 ml by distilled water and then incubated at  $37^\circ\text{C}$  for 24 h. Finally, Nelson reagent was stored by dark bottle.”

### 1.3 Standard glucose

0.02 g glucose was dissolved by distilled water and adjusted to 100 ml. Lastly, 200  $\mu\text{g}/\text{ml}$  will be obtained. It was diluted to various concentration of 50, 100, 150  $\mu\text{g}/\text{ml}$ .



Blank is water

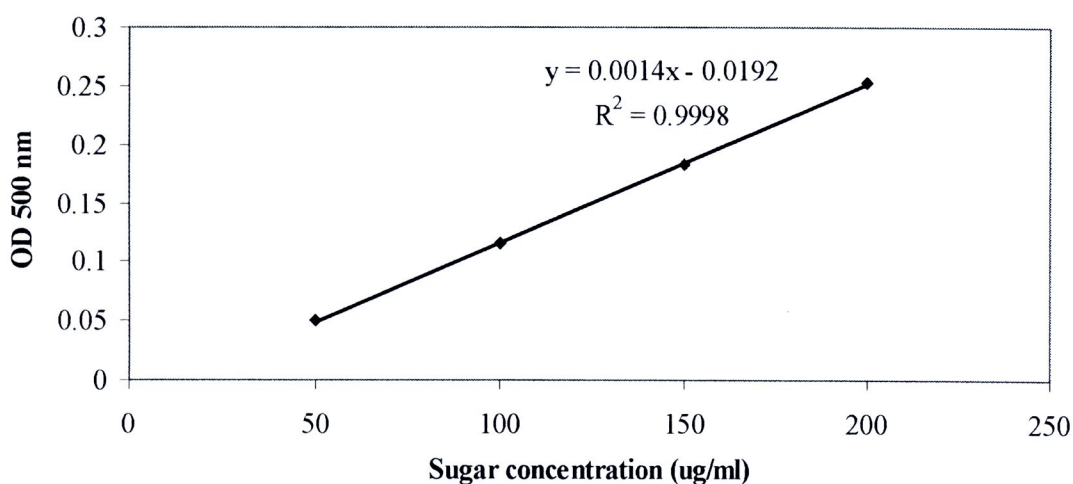


Figure B1. Standard curve of total sugar content (as glucose) at  $\text{OD}_{500}$  nm determined by Somogji-Nelson assay.

## 2. HPLC assay of reducing sugars

**Condition:** Mobile phase: acetonitrile (ACN) and HPLC water (v/v) (75:25 v/v)

Injection: 20  $\mu$ l

Column: Zorbax NH<sub>2</sub> column (4.6 $\times$ 250 mm, 5-Micron, Agilent, USA)  
with RI detector

Operation temperature and flow rate: 25°C, flow rate of 0.7 ml min<sup>-1</sup>

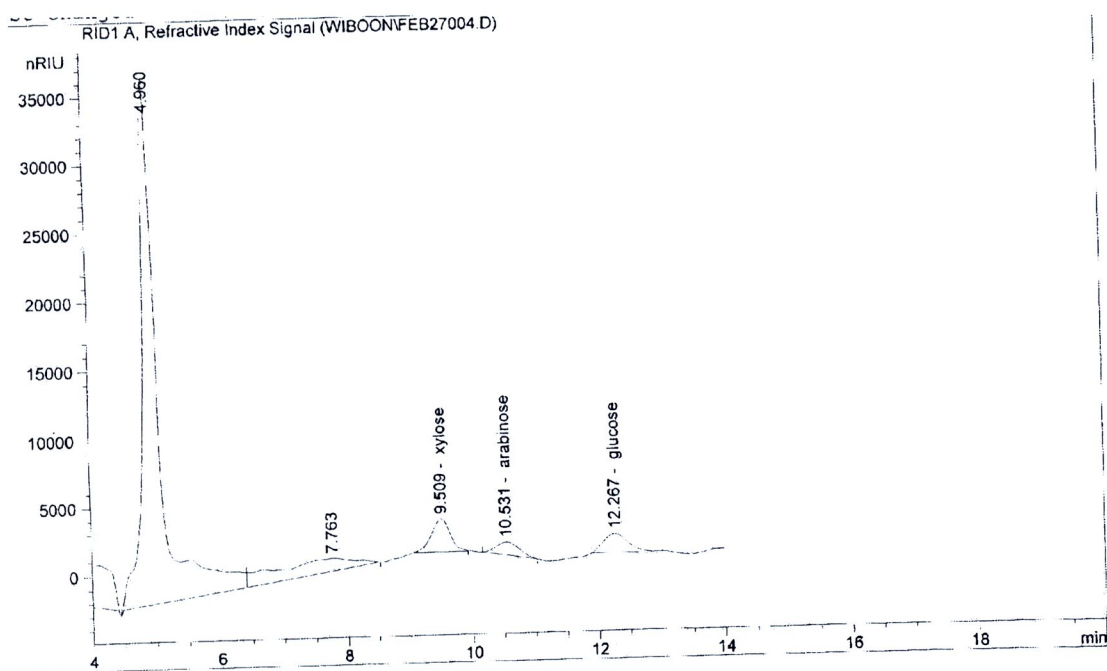


Figure B2. Standard curve of xylose, arabinose, and glucose determined by HPLC.

## Appendix C

### Protein analysis and standard curve

#### 1. Chemical reagents

- 1) Reagent A: 2% (w/v)  $\text{Na}_2\text{CO}_3$  in 0.1 N NaOH.
- 2) Reagent B: 0.5% (w/v)  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 1% sodium potassium tartrate.
- 3) Reagent C: 30 ml reagent A is mixed by 2 ml reagent B.
- 4) Folin-ciocatus phenol reagent is diluted by distilled water in the ratio of 1:2.
- 5) Bovine Serum Albumen (BSA) 1 mg/ml

#### 2. Method

0.5 ml BSA (0, 20, 40, 60, 80 and 100  $\mu\text{g}/\text{ml}$ ) and samples were added  
in each test tube

⇓

Add 0.5 ml reagent A and boiled for 5 min and cooled by ice

⇓

Add 2.5 ml reagent C and mixed well and incubated at room temperature for 10 min

⇓

Add Folin reagent 0.5 ml and mixed well and incubated at room temperature for 30  
min

⇓

Measure OD at 750 nm

#### 3. Curve of standard protein

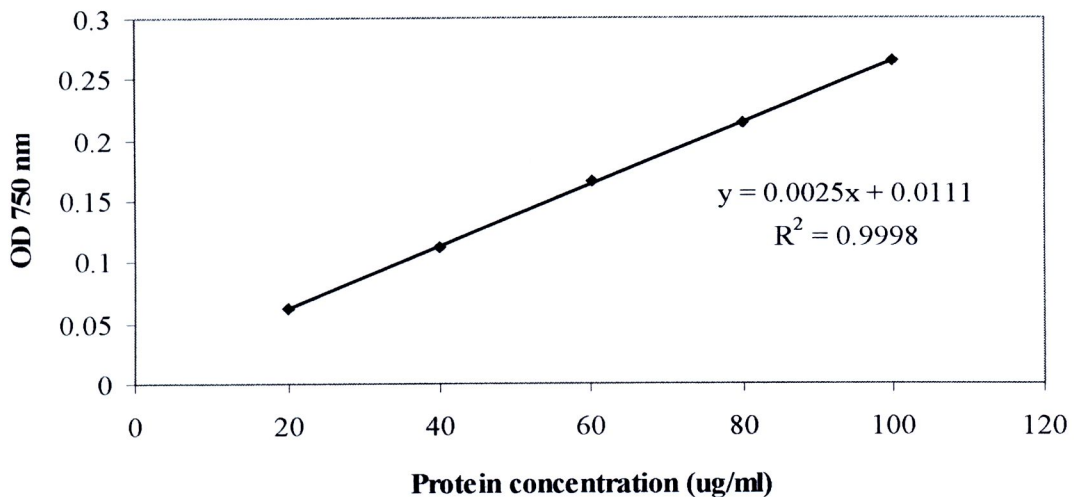


Figure C. Standard curve of protein as BSA at  $\text{OD}_{750 \text{ nm}}$ .

## Appendix D

### Furfural and acetate analysis and standard curves

Furfural and acetate were determined by Gas Chromatography (GC) assay.

**Condition:** Injection: 1  $\mu$ l

Column: Stabilwax®-DA, Restek GC columns

Operation temperature at injection and detector port: 230 and 250°C

Oven: hold at 70°C for 3 min, (ii) ramp for 5 min at 20°C min<sup>-1</sup> to a final temperature of 235°C, (iii) hold at 235°C for 3 min.

Carrier gas: Helium at flow rate 1.2 ml min<sup>-1</sup>.

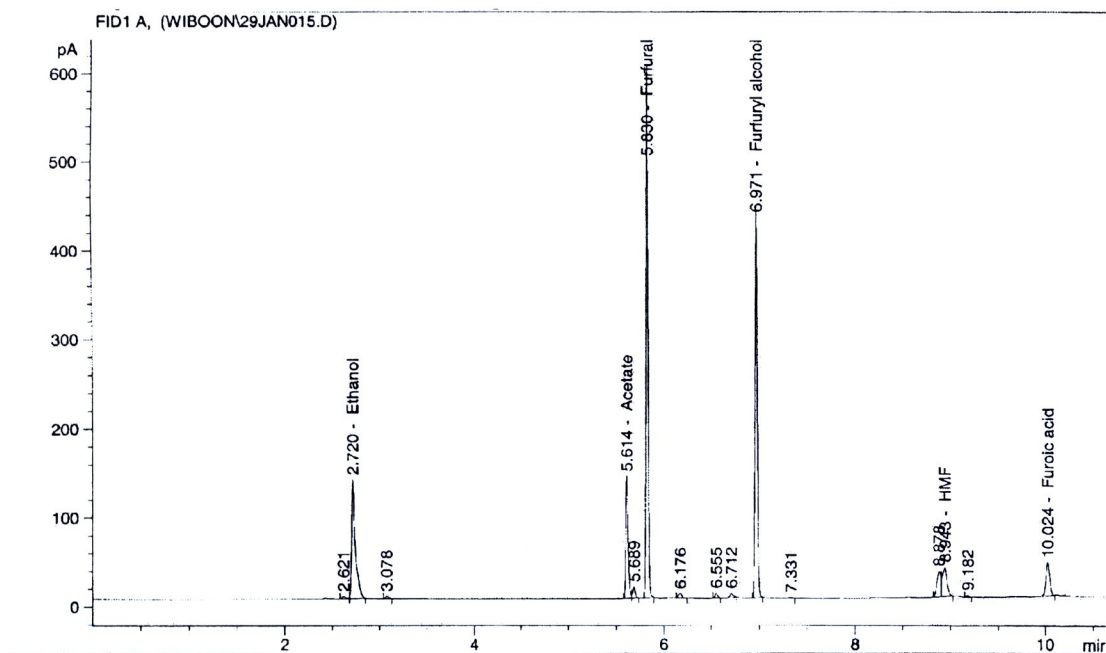


Figure D. Standard curve of ethanol, acetate, furfural, and furfuryl alcohol determined by GC.

## Appendix E

### Dry cell weight

1. The culture of *C. shehatae* TISTR5843, cultured under at 30°C for 24 h, was adjusted the turbidometrically at the wavelength of 600 nm (OD<sub>600</sub>) to be 0.2-1.0.
2. Cell pellet was harvested and twice washed with distilled water by centrifuge (8,000 xg for 10 min).
3. Cell pellet remaining in tubes were baked at 105°C for 12 h. and weighted the dry cell until their constantly weight.

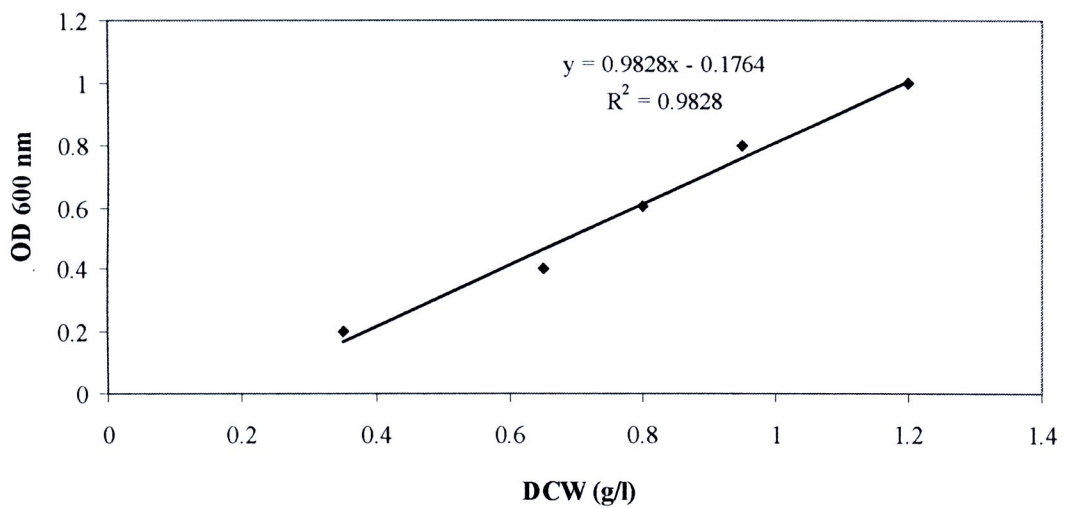


Figure E. Standard curve of dry cell weight of *C. shehatae* TISTR5843.

## Appendix E

### 1. Calculation of glucose production

$$\text{The ratio of dPPF to sulfuric acid} = \frac{1 \text{ g dPPF}}{10 \text{ ml}}$$

$$\text{When, glucose production} = 1.32 \text{ g/l}$$

$$\text{Thus, solution 1000 ml contain glucose } 1.32 \text{ g}$$

$$\text{If solution 10 ml contain glucose} = \frac{1.32 \times 10}{1000}$$

$$= 0.0132 \text{ g glucose /10 ml}$$

From Table 9,

$$1 \text{ g dPPF contain cellulose } 0.42 \text{ g calculated to } 100 \%$$

$$\text{If glucose } 0.0132 \text{ g calculated to } \% = \frac{0.0132 \times 100}{0.42}$$

$$= 3.14 \%$$

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The Palm Oil Products and Technology Research Center (POPTEC), Faculty of Agro-Industry, Prince of Songkla University

### List of Publications and Proceedings

#### Publications

**Riansa-ngawong, W.** and Prasertsan, P. 2010. Statistical approach for optimization of hemicellulose production from delignified palm pressed fiber and used as a bio-material for one-stage production of furfural. *Int. J. Biotechnol. Biochem.* 6(7): 1101-1116.

**Riansa-ngawong, W.** and Prasertsan, P. 2011. Optimization of furfural production from hemicellulose extracted from delignified palm pressed fiber using two-stage process. *Carbohydr. Res.* 346(1): 103-110.

#### Presentations

**Riansa-ngawong, W.**, Prasertsan, P. and Iiyama, K. 2008. Statistical studies on hemicellulose production from delignified palm pressed fiber. *Proceeding of International Biotechnology Symposium (IBS) on October 12-17, 2008, Dalian, China (Poster Presentation).*

- Prasertsan, P., **Riansa-ngawong, W.** and Iiyama, K. 2008. Furfural Production from Hemicelluloses of Delignified Palm Pressed Fiber using Two-stage Process. The 4<sup>th</sup> European Bioremediation Conference 2008 Chania, Crete, Greece (Poster Presentation).
- Riansa-ngawong, W.** and Prasertsan, P. 2009. Ethanol production from hydrolysate of delignified palm pressed fiber by *Candida shehatae* TISTR5843. The 3<sup>rd</sup> International Conference on Fermentation Technology for Value Added Agricultural Products (FerVAAP2009) on August 26-28, 2009, Khon Kaen, Thailand (Oral Presentation).



