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APPENDICES

Appendix A Analytical Procedures

1. Detergent analysis

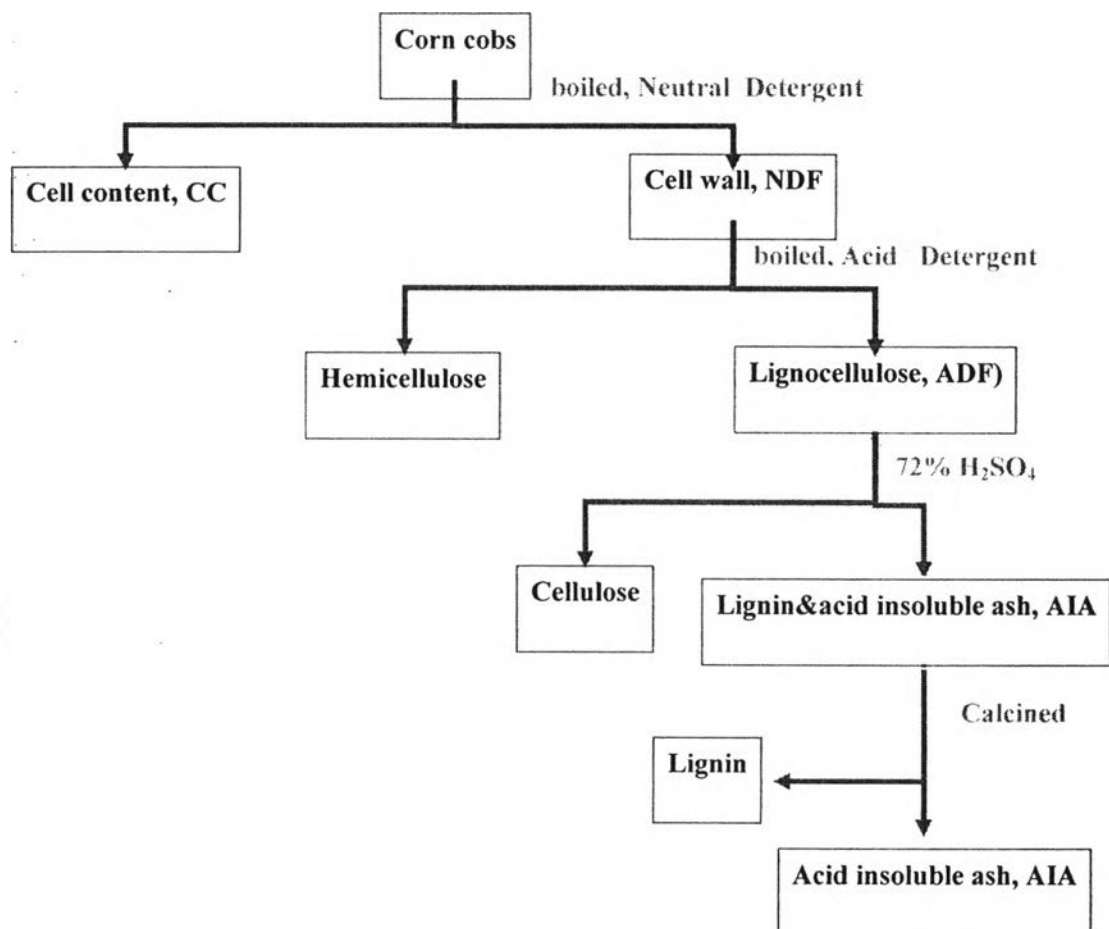


Figure A1 Schematic of detergent analysis procedure flow diagram.

1.1 Neutral detergent fiber (NDF)

Neutral Detergent Fiber (NDF) is cell wall constituents which are not dissolve in neutral solution. NDF consists of hemicellulose, cellulose, lignin, cutin, and keratin.

Reagents

1. Distilled or deionized water
2. Acetone (AR grade)
3. Sodium sulphite
4. Neutral detergent

Procedures

1. Dry crucible at 100 °C for 1 h, after that cool to room temperature at desiccators, and then weigh (W_1).
2. Weigh 1.02 g of dried sample and weigh (W_2).
3. Add 0.5 g of sodium sulphite into crucible.
4. Place crucible from no.3 in Hot Extraction Unit 1020
5. Add 100 mL of neutral detergent into column and reflux for 60 minutes.
6. Rinse sample into crucible with minimum of hot (90°C–100°C) water until the pH of solution reach neutral pH.
7. Wash sample in crucible with 25 mL of acetone for 3 times.
8. Dry sample at 100 °C for 5 h, after that cool to room temperature at desiccators, and then weigh (W_3).
9. Ash residue in crucible for 2 h at 550 °C, after that cool to room temperature at desiccators, and then weigh (W_4).
10. Determine %NDF:

$$\%NDF = \frac{W_4 - W_3}{W_2} \times 100$$

1.2 Acid detergent fiber (ADF)

Acid detergent fiber (ADF) is cell wall constituents which are not dissolve in acid solution. ADF mainly consists of cellulose and lignin, and small amount of cutin. Therefore, loss of product in acid solution should be hemicellulose.

Reagents

1. Distilled or deionized water
2. Acetone (AR grade)
3. Sulfuric acid (H₂SO₄)

Procedures

1. Dry crucible at 100 °C for 1 h, after that cool to room temperature at desiccators, and then weigh (W₁).
2. Weigh 1.02 g of dried sample and weigh (W₂).
3. Add 0.5 g of sodium sulphite into crucible.
4. Place crucible from no.3 in Hot Extraction Unit 1020
5. Add 100 mL of acid detergent into column and reflux for 60 minutes.
6. Rinse sample into crucible with minimum of hot (90°C–100°C) water until the pH of solution reach neutral pH.
7. Wash sample in crucible with 25 mL of acetone for 3 times.
8. Dry sample at 100 °C for 5 h, after that cool to room temperature at desiccators, and then weigh (W₃).
9. Ash residue in crucible for 2 h at 550 °C, after that cool to room temperature at desiccators, and then weigh (W₄).
10. Determine %ADF:

$$\%ADF = \frac{W_4 - W_3}{W_2} \times 100$$

11. Determine % Hemicellulose:

$$\%Hemicellulose = \%NDF - \%ADF$$

1.3 Acid detergent lignin (ADL)

Acid detergent lignin (ADL) is cell wall constituents which are not dissolve in acid solution. ADF mainly consists of cellulose and lignin, and small amount of cutin. Therefore, loss of product in acid solution should be hemicellulose.

Reagents

1. Distiled or deionized water
2. 72% Sulfuric acid (H₂SO₄)

Procedures

1. Fill crucible, which contains the sample from ADF analytical, about half full with 72% H₂SO₄ and stir.
2. Refill with 72% H₂SO₄ and stir at hourly intervals as acid drains away.
3. After 3 h, filter off as much acid as possible with vacuum and wash contents with hot water until free from acid.
4. Dry sample at 100 °C for 5 h, after that cool to room temperature at desiccators, and then weigh (W₃).
5. Ignite crucible in muffle furnace for 2 h at 550 °C, after that cool to room temperature at desiccators, and then weigh (W₄).
6. Determine %Lignin:

$$\%ADL = \frac{W_4 - W_3}{W_2} \times 100$$

7. Determine % Cellulose:

$$\%Cellulose = \%ADF - \%ADL$$

2. Monosaccharide analysis

Monosaccharide mainly consists of glucose, xylose, arabinose. The soluble sugars in the liquid fraction of process samples can be quantified by HPLC with refractive index detection.

Reagents

1. Distilled or deionized water
2. High purity sugars for standards - glucose, xylose, and arabinose

Procedures

1. Prepare a series of sugar calibration standards in HPLC grade water at concentrations appropriate for creating a calibration curve for each sugar of interest.
2. Prepare a set of multi-component standards containing glucose, xylose, and arabinose in the range of 0.2 - 20.0 g/L.
3. Analyze the calibration standards, the calibration verification standards, and the samples by HPLC using a Lichrospher column for glucose, xylose, and arabinose. The following instrumental conditions are used for the Lichrospher column:

Sample volume: 10 μ L.

Eluant: 0.45 μ m filtered and degassed HPLC grade water.

Flow rate: 1.6 mL/min.

Column temperature: 25 °C.

Detector: refractive index.

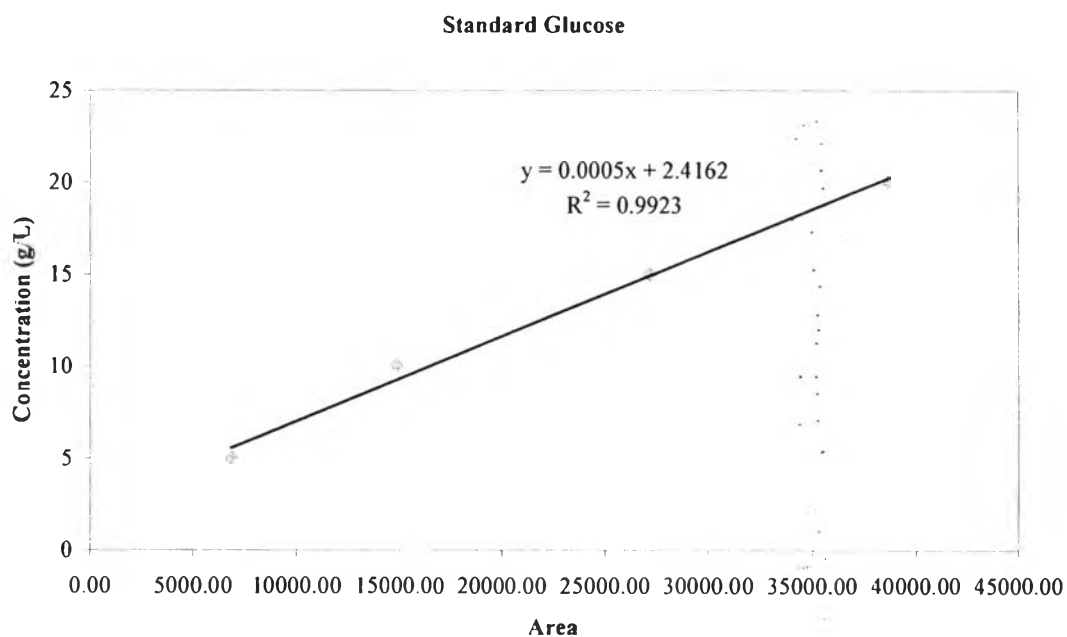
Run time: 20 minutes data collection plus a 15 minute post-run.

4. The retention time of standard was shown in the following:

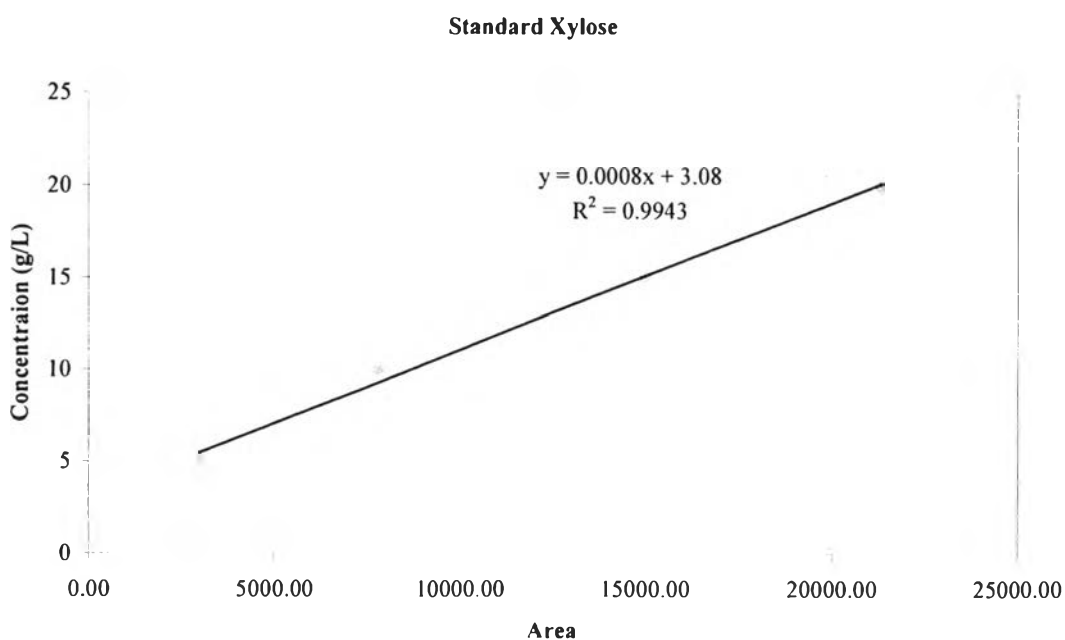
| Standard | Lichrospher column (minutes) |
|-----------|------------------------------|
| Glucose | 9.633 |
| Xylose | 5.466 |
| Arabinose | 6.433 |

5. Create a calibration curve for each sugar to be quantified using linear regression. From these curves as show in Figure A2, determine the concentration in g/L of the sugars present in each sample analyzed by HPLC, corrected for dilution.

(a)



(b)



(c)

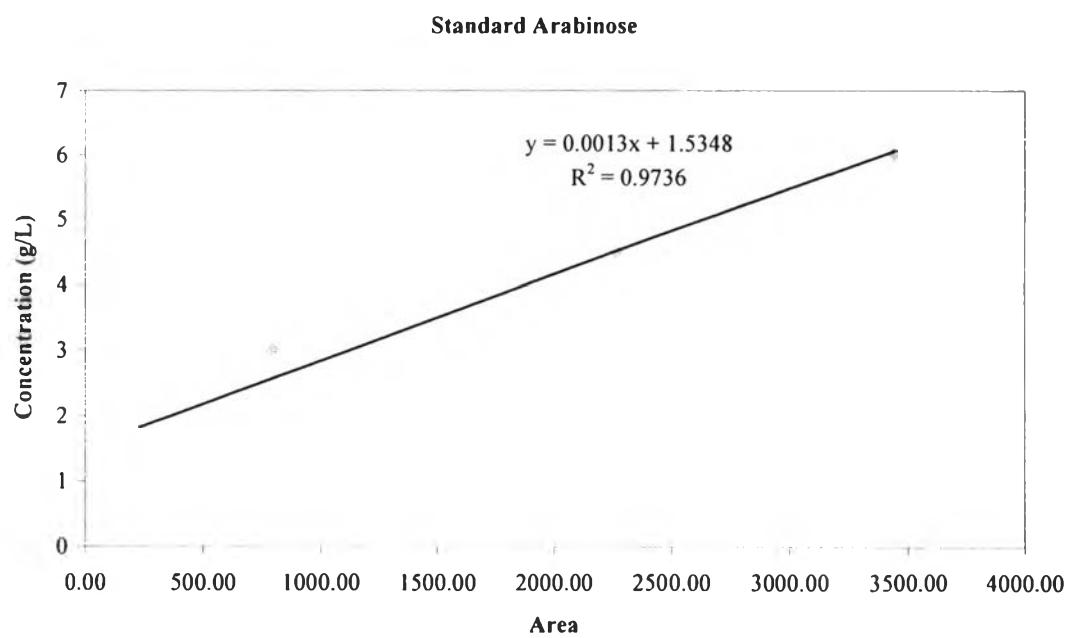


Figure A2 Calibration curve for each sugar: (a) glucose, (b) xylose, and (c) arabinose.

Appendix B Experimental Data for Chapter 4

Table B1 Glucose produced from enzymatic hydrolysis of pretreated corn cobs.

| %NaOH | time(min) | Glucose (g/L) | | | |
|-------|-----------|---------------|----------|----------|----------|
| | | 60 °C | 80 °C | 100 °C | 120 °C |
| 0 | 5 | 10.97833 | 11.42734 | 13.23152 | 11.50535 |
| 0 | 10 | 11.14087 | 11.13767 | 11.3161 | 11.67667 |
| 0 | 20 | 12.27033 | 11.58978 | 12.88947 | 12.68416 |
| 0 | 30 | 12.33564 | 11.9771 | 11.91532 | 12.26595 |
| 0 | 40 | – | – | 11.70928 | – |
| 0.75 | 5 | 13.47159 | 16.553 | 20.49042 | 21.22237 |
| 0.75 | 10 | 14.15067 | 16.28962 | 24.07288 | 25.98327 |
| 0.75 | 20 | 14.8599 | 16.52168 | 24.52326 | 24.35899 |
| 0.75 | 30 | 14.13765 | 21.42968 | 24.94504 | 21.91762 |
| 0.75 | 40 | – | – | 24.43809 | – |
| 1 | 5 | 13.77719 | 17.6791 | 25.5316 | 29.06272 |
| 1 | 10 | 14.98617 | 19.27712 | 26.28241 | 28.18394 |
| 1 | 20 | 15.83114 | 17.17987 | 27.16986 | 25.04244 |
| 1 | 30 | 16.77431 | 21.80783 | 28.22819 | 22.65706 |
| 1 | 40 | – | – | 27.62337 | – |
| 2 | 5 | 16.27278 | 22.75211 | 28.50464 | 25.88157 |
| 2 | 10 | 16.30215 | 23.18901 | 27.11069 | 28.43364 |
| 2 | 20 | 19.94341 | 24.33816 | 30.58381 | 28.32021 |
| 2 | 30 | 20.43323 | 26.15164 | 32.52091 | 24.57433 |
| 2 | 40 | – | – | 30.53125 | – |

| %NaOH | time(min) | Glucose (g/L) | | | |
|-------|-----------|---------------|----------|----------|----------|
| | | 60 °C | 80 °C | 100 °C | 120 °C |
| 3 | 5 | 16.83793 | 18.03171 | 23.43332 | 28.69463 |
| 3 | 10 | 19.11521 | 21.4888 | 24.12222 | 29.00808 |
| 3 | 20 | 20.58978 | 27.58698 | 30.14113 | 24.89909 |
| 3 | 30 | 20.71136 | 28.79714 | 32.70288 | 21.92731 |
| 3 | 40 | – | – | 31.64322 | – |

Table B2 Total sugar produced from enzymatic hydrolysis of pretreated corn cobs.

| %NaOH | time(min) | Total sugar (g/L) | | | |
|-------|-----------|-------------------|----------|----------|----------|
| | | 60 °C | 80 °C | 100 °C | 120 °C |
| 0 | 5 | 10.97833 | 14.45323 | 13.23152 | 11.50535 |
| 0 | 10 | 11.14087 | 14.16356 | 11.3161 | 11.67667 |
| 0 | 20 | 12.27033 | 14.61568 | 12.88947 | 12.68416 |
| 0 | 30 | 12.33564 | 15.00299 | 11.91532 | 12.26595 |
| 0 | 40 | – | – | 11.70928 | – |
| 0.75 | 5 | 17.70473 | 21.74537 | 30.625 | 33.22271 |
| 0.75 | 10 | 18.378 | 23.23229 | 34.49888 | 39.0267 |
| 0.75 | 20 | 19.41281 | 22.58141 | 35.1195 | 33.15632 |
| 0.75 | 30 | 19.27732 | 30.32762 | 35.74125 | 30.40006 |
| 0.75 | 40 | – | – | 36.37012 | – |
| 1 | 5 | 18.63193 | 23.59948 | 37.13343 | 39.48754 |
| 1 | 10 | 19.696 | 25.33247 | 38.12337 | 40.25582 |
| 1 | 20 | 20.66845 | 23.74201 | 39.17178 | 34.23544 |
| 1 | 30 | 21.83925 | 32.17321 | 40.69488 | 30.96744 |
| 1 | 40 | – | – | 40.9058 | – |

| %NaOH | time(min) | Total sugar (g/L) | | | |
|-------|-----------|-------------------|----------|----------|----------|
| | | 60 °C | 80 °C | 100 °C | 120 °C |
| 2 | 5 | 23.34057 | 30.90422 | 40.04875 | 36.25031 |
| 2 | 10 | 20.93682 | 34.6932 | 39.44466 | 39.7752 |
| 2 | 20 | 26.04626 | 33.05928 | 42.85034 | 37.03061 |
| 2 | 30 | 26.80584 | 36.66956 | 45.60271 | 34.30326 |
| 2 | 40 | – | – | 44.71757 | – |
| 3 | 5 | 23.15645 | 23.18557 | 31.73111 | 38.96563 |
| 3 | 10 | 24.45071 | 30.23255 | 33.06957 | 38.97319 |
| 3 | 20 | 26.43469 | 35.52239 | 40.43325 | 31.43203 |
| 3 | 30 | 26.44558 | 40.71544 | 42.98738 | 27.94916 |
| 3 | 40 | – | – | 43.54283 | – |

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1. Ploypradith, P., Luengnaruemitchai, A., and Wongkasemjit, S. (2011, April 26) Pretreatment by Microwave/alkali of Corn Cobs for Butanol Production. Proceedings of The 2nd Research Symposium on Petroleum, Petrochemicals, and Advanced Material and The 17th PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.