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APPENDICES

Appendix A Analytical Procedures

1. Detergent Analysis

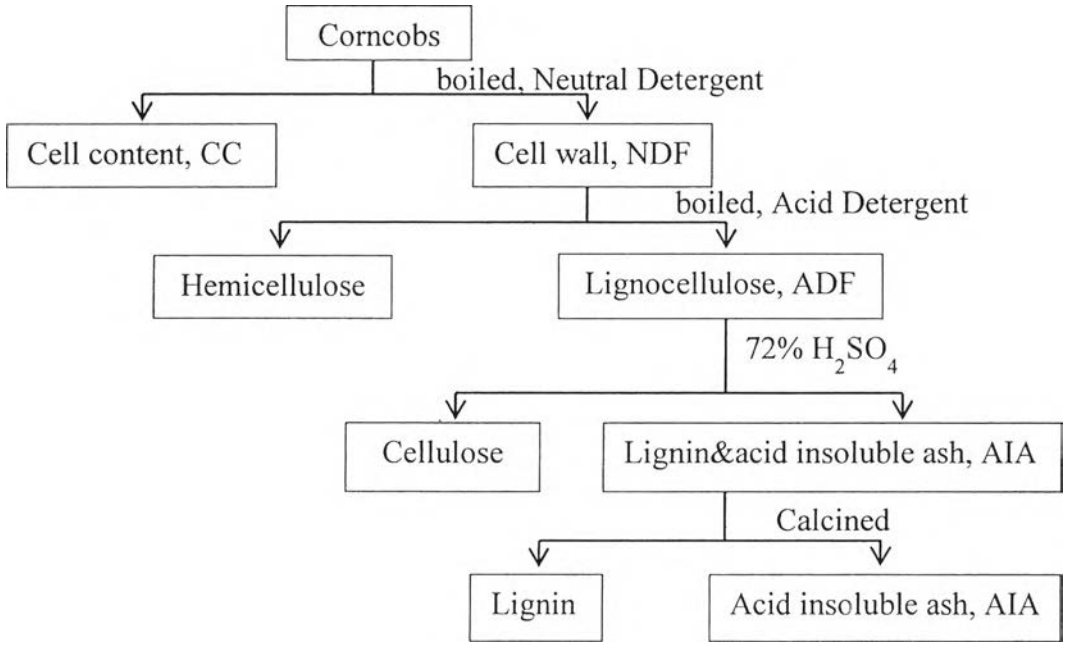

Figure A1 Schematic of detergent analysis procedure flow diagram.

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.

1.1.1 Reagents

1.1.1.1 Distiled or deionized water

1.1.1.2 Acetone (AR grade)

1.1.1.3 Sodium sulphite

1.1.1.4 Neutral detergent

1.1.2 Procedures

1.1.2.1 Dry crucible at 100 °C for 1 h, after that cool to room temperature at desiccators, and then weigh (W_1).

1.1.2.2 Weigh 1.02 g of dried sample and weigh (W_2).

1.1.2.3 Add 0.5 g of sodium sulphite into crucible.

1.1.2.4 Place crucible from no.3 in Hot Extraction Unit 1020

1.1.2.5 Add 100 ml of neutral detergent into column and reflux for 60 min.

1.1.2.6 Rinse sample into crucible with minimum of hot (90 °C–100 °C) water until the pH of solution reach neutral pH.

1.1.2.7 Wash sample in crucible with 25 ml of acetone

1.1.2.8 Dry sample at 100 °C for 5 h, after that cool to room temperature at desiccators, and then weigh (W_3).

1.1.2.9 Ash residue in crucible for 2 h at 550 °C, after that cool to room temperature at desiccators, and then weigh (W_4).

1.1.3 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.

1.2.1 Reagents

1.2.1.1 Distiled or deionized water

1.2.1.2 Acetone (AR grade)

1.2.1.3 Sulfuric acid (H_2SO_4)

1.2.2 Procedures

1.2.2.1 Dry crucible at 100 °C for 1 h, after that cool to room temperature at desiccators, and then weigh (W_1).

1.2.2.2 Weigh 1.02 g of dried sample and weigh (W_2).

1.2.2.3 Add 0.5 g of sodium sulphite into crucible.

1.2.2.4 Place crucible from no.3 in Hot Extraction Unit 1020

1.2.2.5 Add 100 ml of acid detergent into column and reflux for 60 min.

1.2.2.6 Rinse sample into crucible with minimum of hot (90 °C–100 °C) water until the pH of solution reach neutral pH.

1.2.2.7 Wash sample in crucible with 25 ml of acetone for 3 times.

1.2.2.8 Dry sample at 100 °C for 5 h, after that cool to room temperature at desiccators, and then weigh (W_3).

1.2.2.9 Ash residue in crucible for 2 h at 550 °C, after that cool to room temperature at desiccators, and then weigh (W_4).

1.2.3 Determine % ADF

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

1.2.4 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.

1.3.1 Reagents

1.3.1.1 Distiled or deionized water

1.3.1.2 72 % Sulfuric acid (H_2SO_4)

1.3.2 Procedures

1.3.2.1 Fill crucible, which contains the sample from ADF analytical, about half full with 72 % H_2SO_4 and stir.

1.3.2.2 Refill with 72 % H_2SO_4 and stir at hourly intervals as acid drains away.

1.3.2.3 After 3 h, filter off as much acid as possible with vacuum and wash contents with hot water until free from acid.

1.3.2.4 Dry sample at 100 °C for 5 h, after that cool to room temperature at desiccators, and then weigh (W_3).

1.3.2.5 Ignite crucible in muffle furnace for 2 h at 550 °C, after that cool to room temperature at desiccators, and then weigh (W_4).

1.3.3 Determine % Lignin

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

1.3.4 Determine % Cellulose

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

Appendix B Remaining sugars and ABE concentration from 8 techniques of ABE fermentation

Table B1 Remaining sugars and ABE concentration from P2 medium technique

(g/l)	Fermentation time (h)					
	0	6	12	24	48	72
Glucose	38.56	35.93	31.95	21.81	8.75	7.59
Acetone	0.04	0.12	1.07	1.97	3.85	3.70
Ethanol	0	0	0.02	0.10	0.18	0.16
Butanol	0.04	0.10	0.94	3.37	6.53	6
Total ABE	0.08	0.22	2.03	5.44	10.56	9.86

Table B2 Remaining sugars and ABE concentration from control (C) technique

(g/l)	Fermentation time (h)					
	0	6	12	24	48	72
Glucose	47.00	52.74	46.94	48.15	46.05	45.93
Xylose	19.69	22.14	19.74	20.11	19.47	19.35
Arabinose	3.38	3.82	3.40	3.65	3.43	3.40
Acetone	0.04	0.03	0.04	0.03	0	0
Ethanol	0	0	0	0	0	0
Butanol	0.03	0.03	0.03	0.03	0.03	0.02
Total ABE	0.07	0.06	0.07	0.06	0.03	0.02

Table B3 Remaining sugars and ABE concentration from 2–stage pretreatment followed by overliming (CO) technique

(g/l)	Fermentation time (h)					
	0	6	12	24	48	72
Glucose	47.63	43.77	44.89	44.37	45.30	44.93
Xylose	20.49	18.81	19.31	19.14	19.47	19.35
Arabinose	4.60	3.86	4.68	3.96	4.52	4.39
Acetone	0.03	0.04	0.04	0.04	0.04	0.03
Ethanol	0	0	0	0	0	0
Butanol	0.03	0.04	0.03	0.04	0.04	0.04
Total ABE	0.06	0.08	0.07	0.08	0.08	0.07

Table B4 Remaining sugars and ABE concentration from 2–stage pretreatment and diluted 2 times of hydrolysate (D2) technique

(g/l)	Fermentation time (h)					
	0	6	12	24	48	72
Glucose	34	35.46	32.74	34.01	31.92	31.23
Xylose	9.33	9.68	9	9.41	9.70	9.67
Arabinose	1.55	1.64	1.50	1.59	1.60	1.58
Acetone	0.04	0.04	0.04	0.03	0.04	0.04
Ethanol	0	0	0	0	0	0
Butanol	0.04	0.05	0.03	0.02	0.03	0.03
Total ABE	0.08	0.09	0.07	0.05	0.07	0.07

Table B5 Remaining sugars and ABE concentration from 2–stage pretreatment followed by overliming and diluted 2 times of hydrolysate (D2O) technique

(g/l)	Fermentation time (h)					
	0	6	12	24	48	72
Glucose	29.30	28.88	27.80	26.59	18.18	17.36
Xylose	11.88	12.05	11.36	10.40	7.56	6.85
Arabinose	2.20	2.15	2.11	2.01	1.69	1.61
Acetone	0.04	0.11	0.34	1.79	2.93	2.69
Ethanol	0	0	0.01	0.05	0.14	0.12
Butanol	0.04	0.06	0.25	1.68	3.08	2.91
Total ABE	0.08	0.17	0.59	3.47	6.15	5.72

Table B6 Remaining sugars and ABE concentration from 2–stage pretreatment and diluted 4 times of hydrolysate (D4) technique

(g/l)	Fermentation time (h)					
	0	6	12	24	48	72
Glucose	20.24	20.95	20.20	18.44	6.29	5.99
Xylose	5.20	5.45	5.28	5.12	3.65	3.56
Arabinose	0.94	0	0	0	0	0
Acetone	0.03	0.07	0.08	0.96	2.09	2.01
Ethanol	0	0	0	0.02	0.23	0.18
Butanol	0.03	0.05	0.05	0.70	2.76	2.32
Total ABE	0.06	0.12	0.13	1.68	5.08	4.51

Table B7 Remaining sugars and ABE concentration from 2–stage pretreatment followed by overliming and diluted 4 times of hydrolysate (D4O) technique

(g/l)	Fermentation time (h)					
	0	6	12	24	48	72
Glucose	20	19.27	18.31	13.94	4.22	3.64
Xylose	6.59	6.47	6.01	5.02	2.86	2.43
Arabinose	1.19	0	0	0	0	0
Acetone	0.04	0.10	0.49	2.14	3.67	3.46
Ethanol	0	0	0.01	0.07	0.15	0.10
Butanol	0.03	0.07	0.32	2.02	4.61	4.41
Total ABE	0.07	0.17	0.82	4.23	8.43	7.97

Table B8 Remaining sugars and ABE concentration from microwave/NaOH followed by water pretreatment (W) technique

(g/l)	Fermentation time (h)					
	0	6	12	24	48	72
Glucose	47.43	45.10	41.01	42.16	39.49	38.84
Xylose	16.60	15.81	14.10	14.02	12.68	11.53
Arabinose	0	0	0	0	0	0
Acetone	0.02	0.10	0.27	1.06	1.48	1.36
Ethanol	0	0	0	0.02	0.04	0.03
Butanol	0.03	0.06	0.15	0.71	1.02	0.91
Total ABE	0.05	0.16	0.42	1.79	2.54	2.20

CURRICULUM VITAE

Name: Ms. Jidapa Manaso

Date of Birth: December 16, 1988

Nationality: Thai

University Education:

2007–2011 Bachelor Degree of Engineering in Chemical Engineering, Faculty of Engineering, King Mongkut's University of Technology North Bangkok. Bangkok, Thailand.

Work Experience:

2010 Position: Internship Student

Company name: The Bangchak Petroleum Company Limited

Proceedings:

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2. Manaso, J.; Luengnaruemitchai, A.; and Wongkasemjit, S. (April 23, 2013) Chemical Pretreatment of Corncobs through Microwave Radiation for Butanol Production. Proceedings of The 4th Research Symposium on Petrochemical, and Material Technology and The 19th PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.