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APPENDICES

Appendix A SEM Images of Sugarcane Bagasse Samples

Figure A1 40 mesh ground bagasse before the hydrolysis

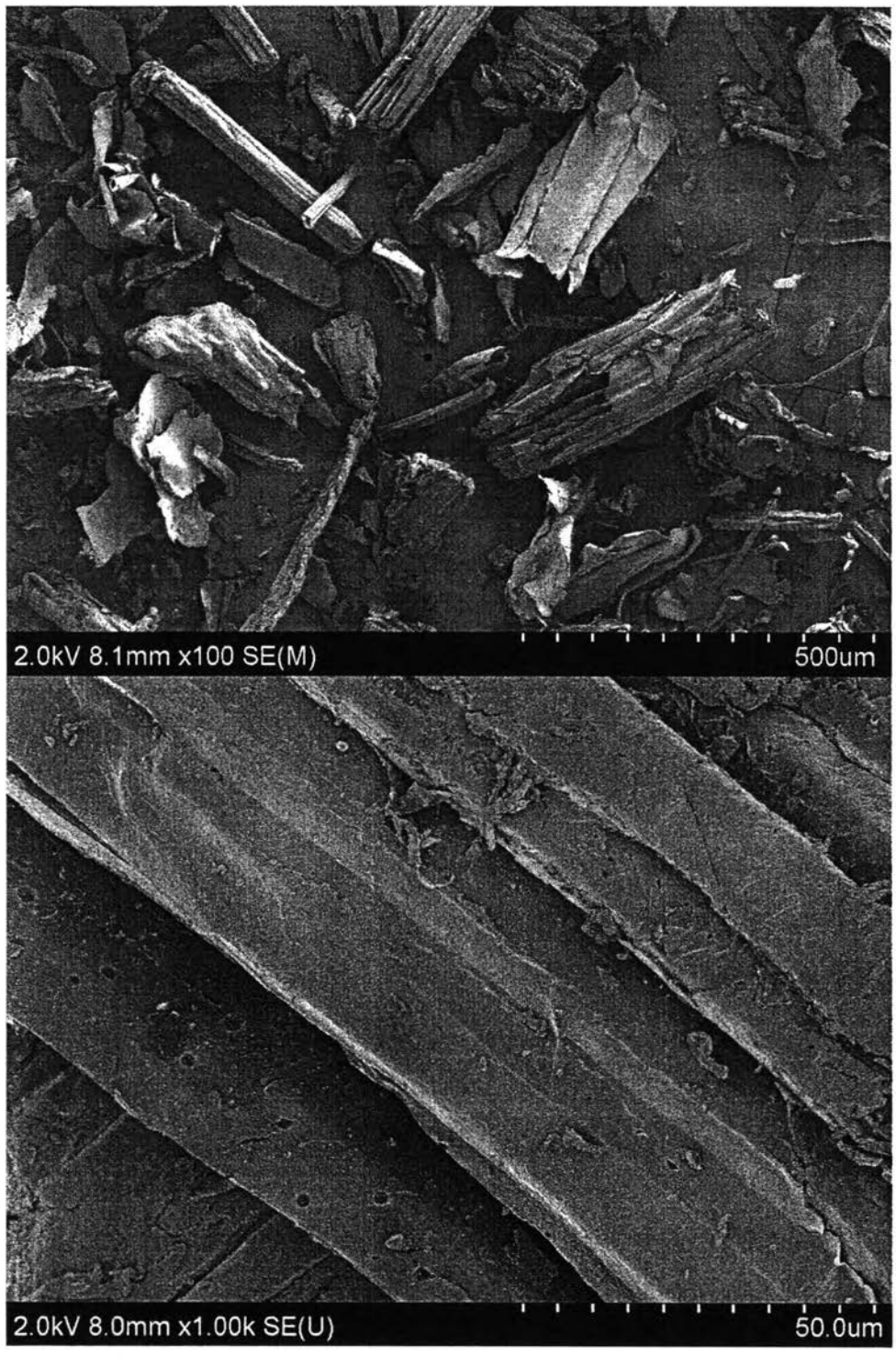

Figure A2 60 mesh ground bagasse before the hydrolysis

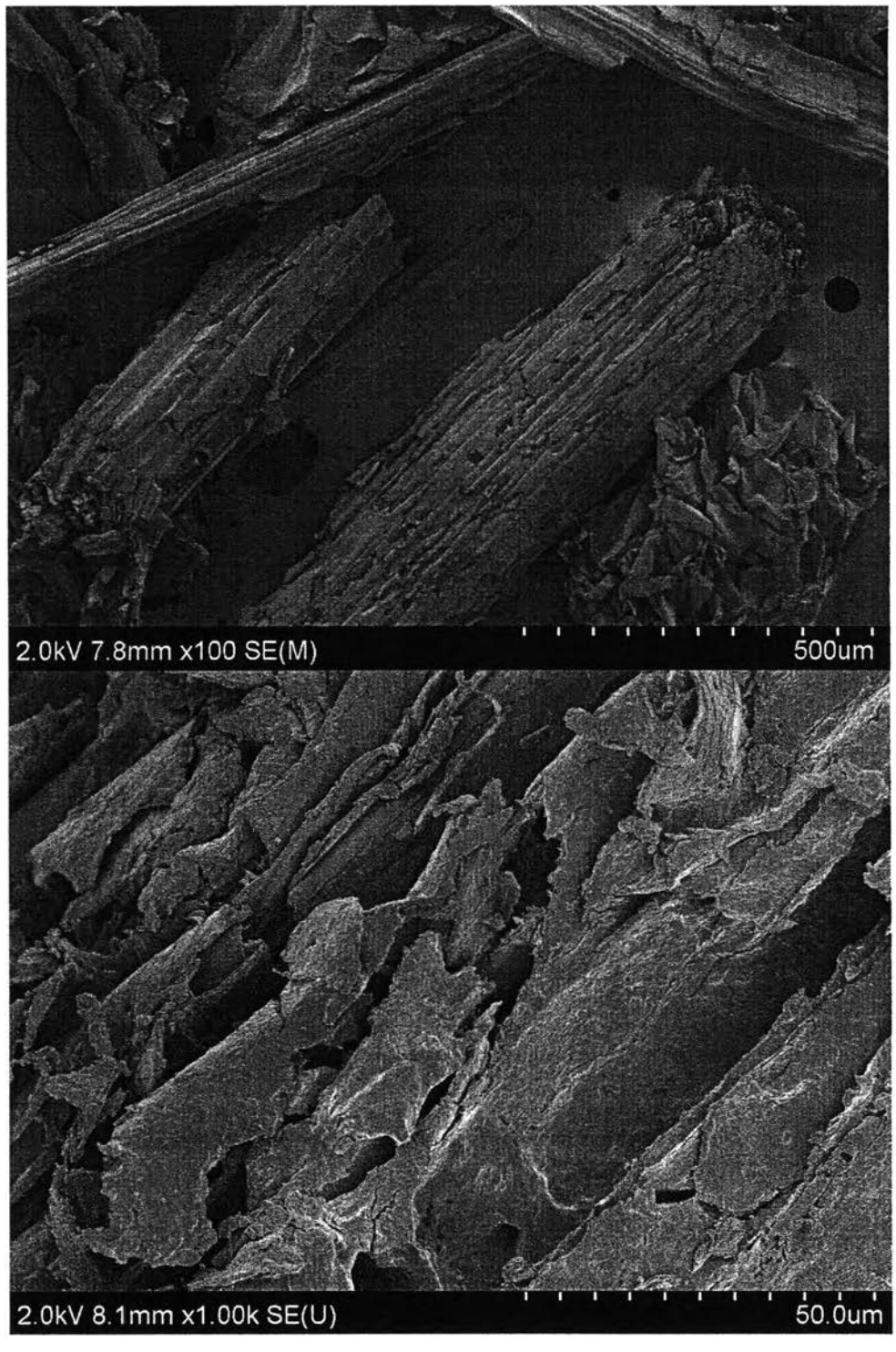

Figure A3 40 mesh ground bagasse after the hydrolysis

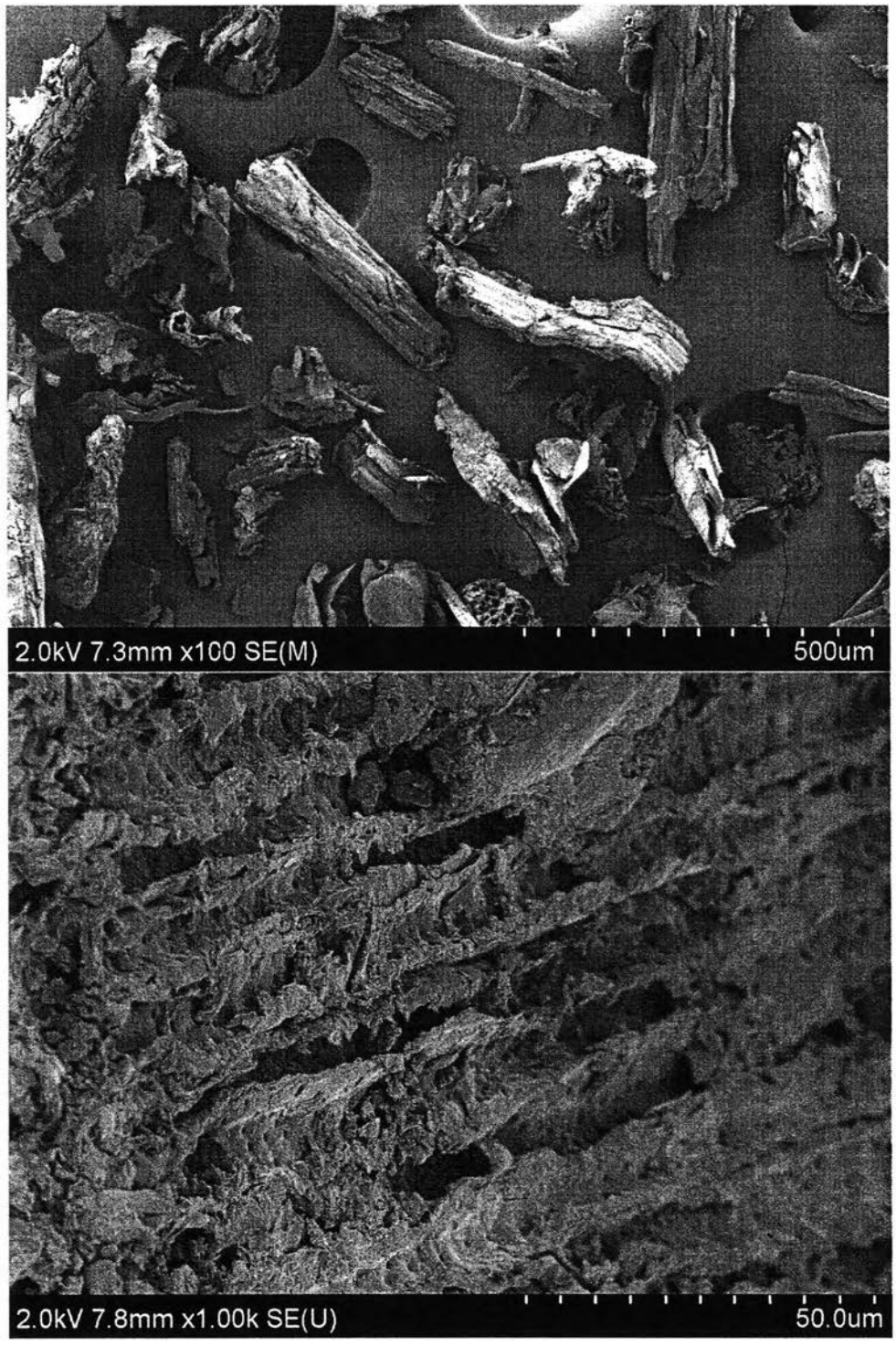

Figure A4 60 mesh ground bagasse after the hydrolysis

Appendix B HPLC Analysis

Standard sample of sugar and alcohol that expected to be in the product to hydrolysis of sugarcane bagasse were analyse to indentify retention time of each product.

Table B1 Retention time of each standard sample

Standard name	Retention time (min)
Sugar	
Cellubiose	22.293
Glucose	26.731
Xylose	28.938
Arabinose	34.856
Mannose	39.072
Galactose	31.720
Alcohol	
Ethanol	33.495
Butanol	33.495

The calibration equation was obtained from calibration graph by using various known concentration of standard sugars and used to calculate for hydrolysis results.

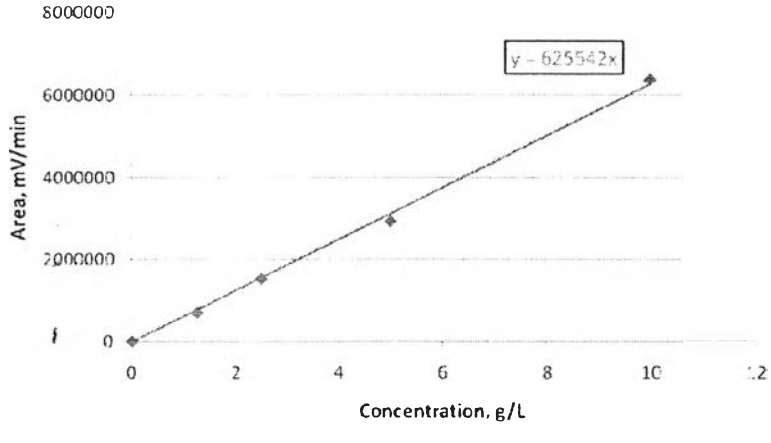

Figure B1 Calibration curve for cellubiose analysis.

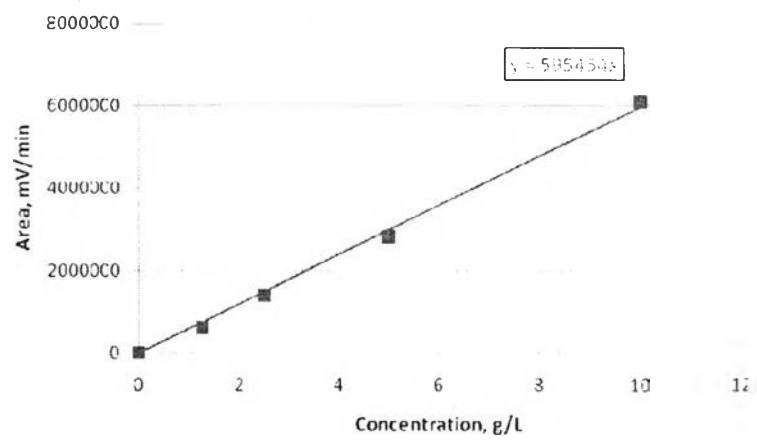

Figure B2 Calibration curve for glucose analysis.

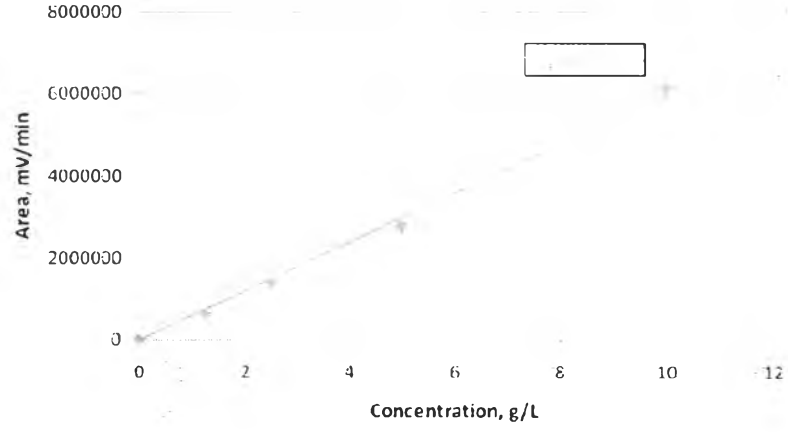

Figure B3 Calibration curve for xylose analysis.

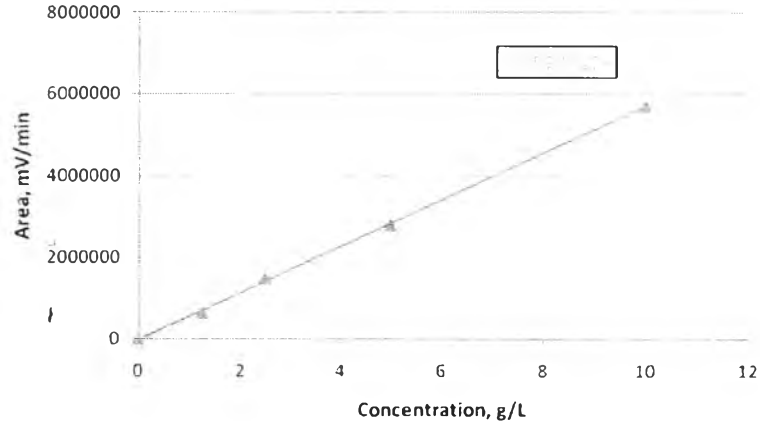

Figure B4 Calibration curve for arabinose analysis.

Appendix C Media for Microorganisms

1. 65 Modified DSMZ Broth Medium 2

Carboxymethyl Cellulose (CMC)	5.0	g
Yeast extract	4.0	g
Malt extract	10.0	g
Distilled water	1000.0	mL

Dissolve and adjust pH to 7.2.

Autoclave at 121°C and 15 psi for 15 min.

2. 65 Modified DSMZ Agar Medium 2

Carboxymethyl Cellulose (CMC)	5.0	g
Yeast extract	4.0	g
Malt extract	10.0	g
Agar	12.0	g
Distilled water	1000.0	mL

Dissolve and adjust pH to 7.2 before adding agar.

Autoclave at 121°C and 15 psi for 15 min.

3. Mineral Nutrient Broth

Carboxymethyl Cellulose (CMC)	5.0	g
Yeast extract	4.0	g
Malt extract	10.0	g
Agar	12.0	g
Distilled water	1000.0	mL

Appendix D Reagent Preparation

1. 0.85%(w/v) NaCl in 1000 mL

Sodium Chloride (NaCl)	8.0	g
Distilled water	1000.0	mL

2. Sodium hydroxide 1 N in 100 mL

Sodium hydroxide (NaOH)	4.0	g
Distilled water	100.0	mL

3. Sodium Hydroxide 0.5 mol/l in 50 mL

Sodium hydroxide (NaOH)	1.0	g
Distilled water	50.0	mL

4. 72%(w/v) Sulfuric Acid in 100 mL

Sulfuric acid (H_2SO_4 conc.)	75	mL
Distilled water	100.0	mL

5. 10%(w/v) Barium Chloride in 100 mL

Barium chloride ($BaCl_2$)	1.0	g
Distilled water	100.0	mL

6. DNS Reagent

Dinitrosalicylic acid	10.0	g
Phenol	2.0	g
Sodium sulfite	0.5	g
Sodium hydroxide	10.0	g
Distilled water	100.0	mL

7. 40% Rochelle Salt Solution

Potassium sodium tartarte	400.0	g
Distilled water	100.0	mL

Appendix E Total N Kit HR (10 – 150 mg N/L) Procedure

The procedure was perfrom as method 10072 from Hach company. Figure E1 shows total nitrogen kit (HR) procedure step.

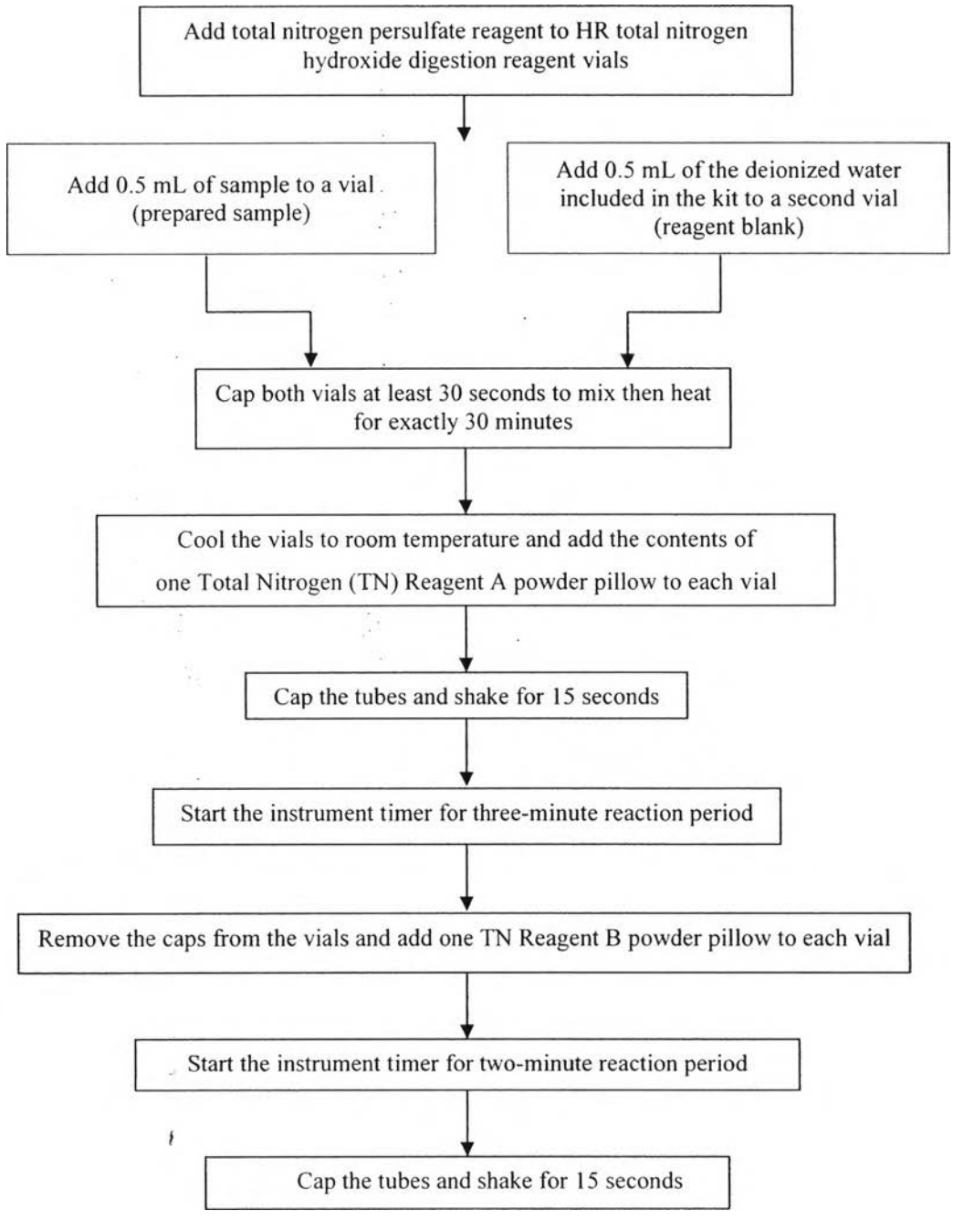

Figure E1 Procedure for analyzing amount of nitrogen.

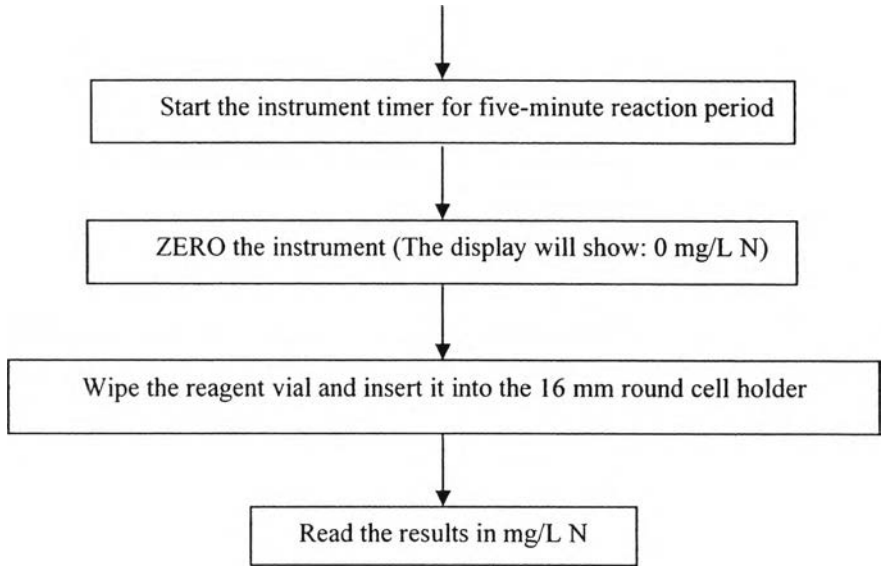

Figure E1 Procedure for analyzing amount of nitrogen (countinued).

Appendix F Experiment Data of Sugarcane Bagasse Hydrolysis

Table G1 Glucose concentration from the hydrolysis of 40 mesh ground bagasse with the M 015 bacteria strain at 37 °C

Time (h)	0	1	2	3	4	5	6	7	10	13	14	20
Glucose g/L)	0.28	0.96	1.06	1.04	1.09	0.9	1.03	0.96	0.79	0.35	0.50	0.50

Table G2 Glucose concentration from the hydrolysis of 60 mesh ground bagasse with the M 015 bacteria strain at 37 °C

Time (h)	0	1	2	3	4	5	6	7	10	13	14	20
Glucose g/L)	0.28	0.79	0.95	1.04	1.13	1.00	0.91	0.86	0.92	0.79	0.82	0.71

Table G3 Glucose concentration from the hydrolysis of 40 mesh ground bagasse with the A 002 bacteria strain at 37 °C

Time (h)	0	1	2	3	5	6	7	8	9	10	11	12	13	15	17	19	23
Glucose g/L)	0.28	0.31	0.24	0.29	0.25	0.26	0.25	0.24	0.30	0.47	0.40	0.17	0.23	0.23	0.30	0.27	0.21

Table G4 Glucose concentration from the hydrolysis of 60 mesh ground bagasse with the A 002 bacteria strain at 37 °C

Time (h)	0	1	2	3	5	6	7	8	9	10	11	12	13	15	17	19	23
Glucose g/L)	0.28	0.32	0.34	0.21	0.31	0.36	0.38	0.40	0.51	0.23	0.22	0.20	0.22	0.23	0.24	0.26	0.24

Table G5 Glucose concentration from the hydrolysis of 40 mesh ground bagasse with the M 015 bacteria strain at 30 °C

Time (h)	0	1	2	3	5	7	8	9	10	11	12	15	18	22
Glucose g/L)	0.28	0.30	0.32	0.27	0.35	0.44	0.47	0.48	0.51	0.50	0.33	0.32	0.32	0.31

Table G6 Glucose concentration from the hydrolysis of 60 mesh ground bagasse with the M 015 bacteria strain at 30 °C

Time (h)	0	1	2	3	5	7	8	9	10	11	12	15	18	22
Glucose g/L)	0.28	0.29	0.31	0.38	0.45	0.46	0.53	0.51	0.49	0.36	0.32	0.33	0.34	0.31

Table G7 Glucose concentration from the hydrolysis of 40 mesh ground bagasse with the A 002 bacteria strain at 30 °C

Time (h)	0	1	3	5	7	9	10	11	12	15	18	23
Glucose g/L)	0.28	0.25	0.23	0.20	0.27	0.38	0.43	0.38	0.33	0.28	0.27	0.26

Table G8 Glucose concentration from the hydrolysis of 60 mesh ground bagasse with the A 002 bacteria strain at 30 °C

Time (h)	0	1	3	4	6	7	9	10	11	12	15	23
Glucose g/L)	0.28	0.23	0.14	0.15	0.18	0.38	0.46	0.47	0.34	0.28	0.26	0.25

Table G9 Bacterial concentration from the hydrolysis of 40 mesh ground bagasse with the M 015 bacteria strain at 37 °C

Time (h)	Nitrogen Concentration (g/L)	Bacterial Concentration (g/L)
0	58	0.73
1	69	0.87
2	101	1.28
4	135	1.71
5	112	1.41
7	119	1.50
10	121	1.53
14	125	1.58
20	124	1.57

Table G10 Bacterial concentration from the hydrolysis of 60 mesh ground bagasse with the M 015 bacteria strain at 37 °C

Time (h)	Nitrogen Concentration (g/L)	Bacterial Concentration (g/L)
0	59	0.75
1	75	0.95
2	91	1.15
5	104	1.31
7	107	1.35
10	111	1.40
14	111	1.40
20	119	1.50

Table G11 Bacterial concentration from the hydrolysis of 40 mesh ground bagasse with the A 002 bacteria strain at 37 °C

Time (h)	Nitrogen Concentration (g/L)	Bacterial Concentration (g/L)
0	42	0.75
1	50	0.89
2	48	0.85
5	51	0.90
8	65	1.15
9	83	1.47
11	98	1.74
15	99	1.76
23	101	1.79

Table G12 Bacterial concentration from the hydrolysis of 60 mesh ground bagasse with the A 002 bacteria strain at 37 °C

Time (h)	Nitrogen Concentration (g/L)	Bacterial Concentration (g/L)
0	41	0.73
1	47	0.83
3	53	0.94
5	54	0.96
7	92	1.63
9	101	1.79
10	97	1.72
12	99	1.76
17	97	1.72
24	102	1.81

Table G13 Bacterial concentration from the hydrolysis of 40 mesh ground bagasse with the M 015 bacteria strain at 30 °C

Time (h)	Nitrogen Concentration (g/L)	Bacterial Concentration (g/L)
0	55	0.69
1	59	0.75
2	78	0.99
3	82	1.04
5	97	1.23
8	102	1.29
9	107	1.35
11	109	1.38
15	115	1.45
22	117	1.48

Table G14 Bacterial concentration from the hydrolysis of 60 mesh ground bagasse with the M 015 bacteria strain at 30 °C

Time (h)	Nitrogen Concentration (g/L)	Bacterial Concentration (g/L)
0	55	0.69
1	75	0.95
2	74	0.93
3	88	1.11
5	96	1.21
8	110	1.39
11	118	1.49
15	116	1.47
22	119	1.50

Table G15 Bacterial concentration from the hydrolysis of 40 mesh ground bagasse with the A 002 bacteria strain at 30 °C

Time (h)	Nitrogen Concentration (g/L)	Bacterial Concentration (g/L)
0	40	0.71
1	49	0.87
3	52	0.92
7	60	1.06
9	76	1.35
10	82	1.45
11	87	1.54
15	91	1.61
18	96	1.70
23	98	1.74

Table G16 Bacterial concentration from the hydrolysis of 60 mesh ground bagasse with the A 002 bacteria strain at 30 °C

Time (h)	Nitrogen Concentration (g/L)	Bacterial Concentration (g/L)
0	40	0.71
1	43	0.76
3	45	0.80
7	63	1.12
9	80	1.42
10	95	1.69
11	97	1.72
15	96	1.70
18	99	1.76
23	99	1.76

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1. Nibhondhratana, C., Rangsunvigit, P., Chavadej, S., Sreethawong, T., and Rengpipat, S. (2011, April 26) Hydrolysis of Sugarcane Bagasse for Sugar Production by Microbes from Thai Higher Termites. Proceedings of The 2nd Research Symposium on Petroleum, Petrochemicals, and Advanced Materials and The 17th PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.