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APPENDICES

Appendix A Standard Calibration Curve

Glucose Calibration Curve

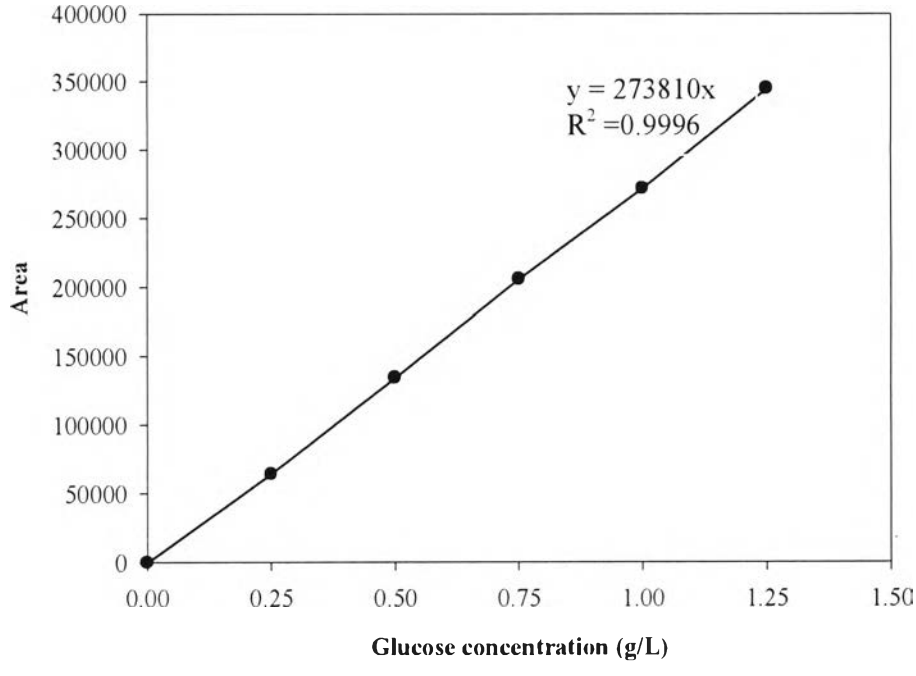

Figure A1 The relationship between glucose concentration (g/L) and area.

Table A1 Glucose calibration curve

Glucose concentration (g/L)	Area(glucose)
0.00	0
0.25	64231
0.50	134469
0.75	206002
1.00	272024
1.25	345123

Appendix B Media for Microorganisms

1. 65 modified DSMZ broth medium 2

Approximate Formula* Per Liter

Carboxymethyl Cellulose (CMC)	5.0	g
Yeast extract	4.0	g
Malt extract	10.0	g

Dissolve and adjust pH to 7.2

Autoclave at 121 °C and pressure at 15 pounds/square inch for 15 minutes

2. 65 modified DSMZ agar medium 2

Approximate Formula* Per Liter

Carboxymethyl cellulose (CMC)	5.0	g
Yeast extract	4.0	g
Malt extract	10.0	g
Agar	12.0	g

Dissolve and adjust pH to 7.2

Autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes

Appendix C Reagent Preparations

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

Sodium chloride (NaCl)	8.5	g
Distilled water	1000	mL

2. Hydrochloric acid 1 M in 100 mL

Hydrochloric acid (HCl)	8.29	mL
Distilled water	91.71	mL

3. Sodium hydroxide 0.5 M in 1000 mL

Sodium hydroxide (NaOH)	5.0	g
Distilled water	1000	mL

4. Sulfuric acid 0.72 M in 1000 mL

Sulfuric acid (H_2SO_4 conc.)	72	mL
Distilled water	28	mL

5. 0.05 M Acetate buffer solution in 1000 mL

0.2 M Acetic acid (CH_3COOH)	7.47	mL
0.2 M Sodium acetate (CH_3COONa)	42.53	mL

6. 0.2 M Acetate acid in 1000 mL

Acetate acid (CH_3COOH)	11.6	mL
Distilled water	988.4	mL

7. 0.2 M Sodium acetate in 1000 mL

Sodium acetate (CH_3COONa)	16.4	mL
Distilled water	983.6	mL

Appendix D Bacteria Concentration

Bacteria concentration was determined using total nitrogen test kit.

1. The bacteria concentration from enzymatic hydrolysis

During enzymatic hydrolysis, bacteria growth was monitored by withdrawing samples from the hydrolysis reactor periodically. Solid that obtained from centrifuging of the sample, contained of corncob and bacteria. Method that can calculate weight of bacteria and corncob is shown in equation D1.

$$\text{wt. Solid} = \text{wt. Corncob} + \text{wt. Bacteria} \qquad \text{(D1)}$$

Then, a concentration of bacteria was determined by the total nitrogen test kit.

$$\text{wt. Bacteria} = \frac{\text{g Nitrogen contained in sample}}{\text{(g Nitrogen / 1 g Bacteria)}} \qquad \text{(D2)}$$

1.1 The amount of nitrogen in bacteria

The amount of nitrogen in each strain was determined in triplicates by using the total nitrogen test kit. Figure F1 shows procedure for determination

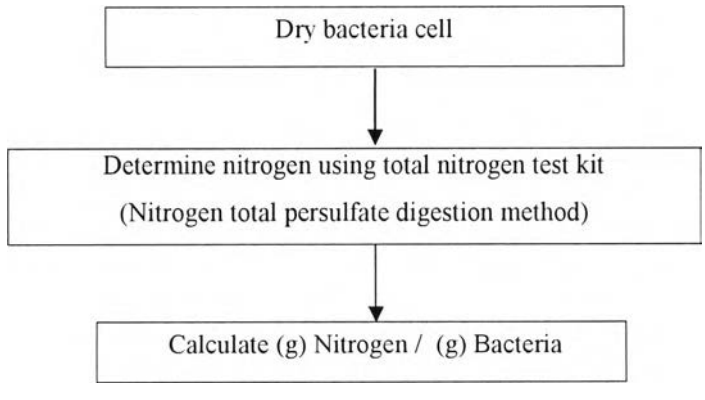

Figure D1 Diagram for determination the amount of nitrogen in bacteria.

Procedure

Nitrogen total persulfate digestion method is conducted in order to check amount of nitrogen which directly related to amount of bacteria during hydrolysis.

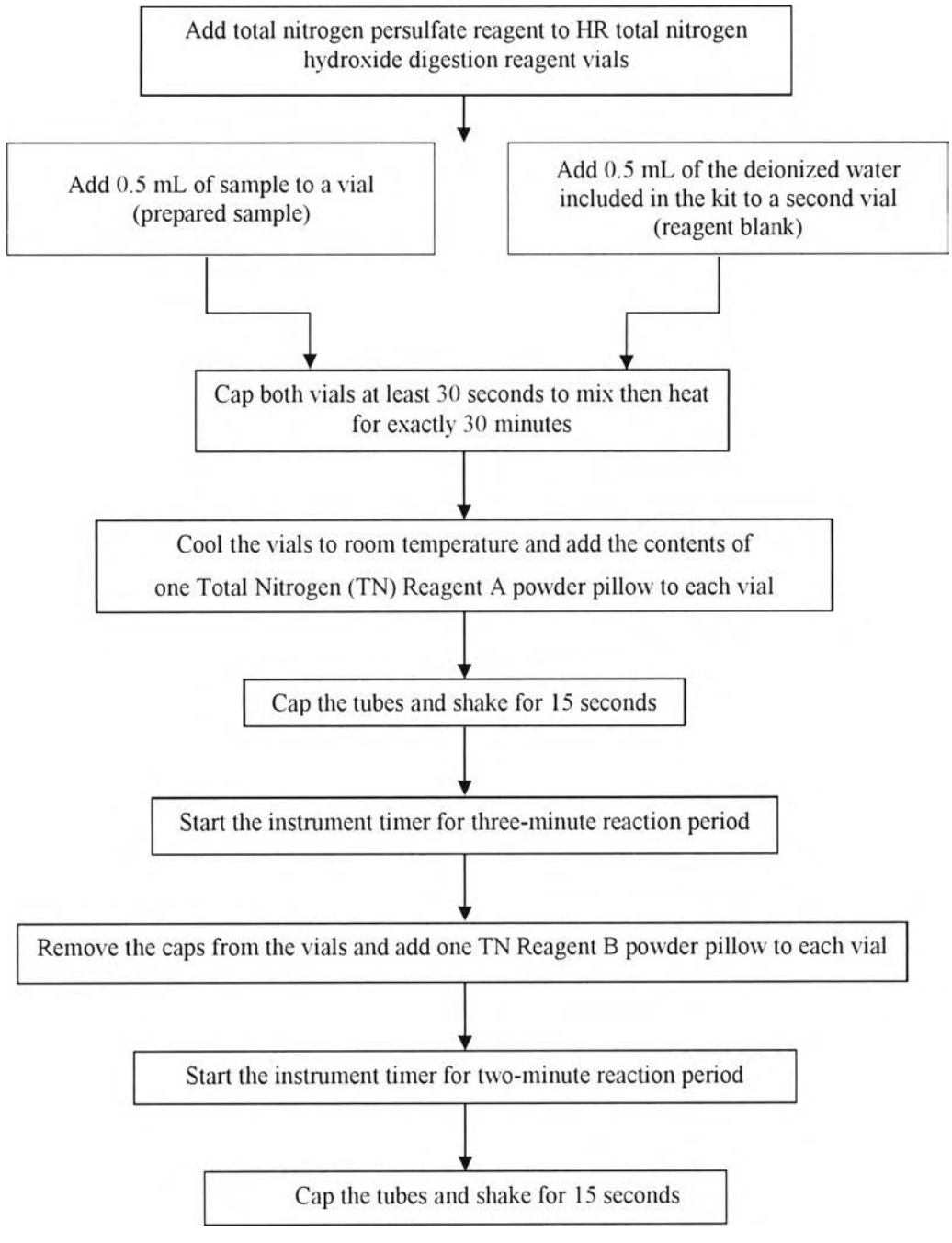

Figure D2 Procedure for analyzing amount of nitrogen.

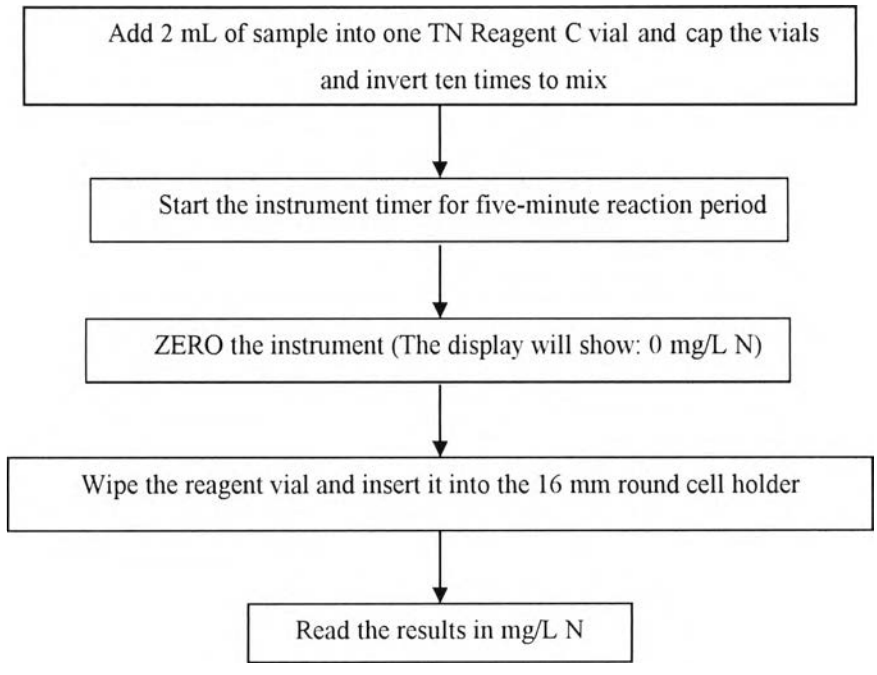

Figure D2 Procedure for analyzing amount of nitrogen (continued).

Appendix E Experiment Data of Hydrolysis

Table E1 Glucose produced from the hydrolysis of 40–60 mesh corncob using the bacterial strain A 002 at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	94161	0.344
1	82901	0.303
2	78151	0.285
3	75478	0.276
4	89106	0.325
5	97523	0.356
6	105926	0.387
8	107257	0.392
10	96622	0.353
12	79008	0.289
16	59124	0.216
20	51846	0.189
24	32039	0.117

Table E2 Glucose produced from the hydrolysis of 60–80 mesh corncob using the bacterial strain A 002 at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	92600	0.338
1	83520	0.305
2	88038	0.322
3	97925	0.358
4	104563	0.382
5	124302	0.454
6	131861	0.482
8	131979	0.482
10	118773	0.434
12	97189	0.355
16	74057	0.270
20	59411	0.217
24	31663	0.116

Table E3 Glucose produced from the hydrolysis of 80–100 mesh corncob using the bacterial strain A 002 at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	93380	0.341
1	22617	0.083
2	48551	0.177
3	97709	0.357
4	121240	0.443
5	141984	0.519
6	165975	0.606
8	130857	0.478
10	107941	0.394
12	77031	0.281
16	13063	0.048
20	12850	0.047
24	13849	0.051

Table E4 Glucose produced from the hydrolysis of 40–60 mesh corncob using the bacterial strain M 015 at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	102361	0.374
1	91097	0.333
2	86275	0.315
3	85746	0.313
4	87061	0.318
5	84585	0.309
6	97794	0.357
8	104094	0.380
10	113949	0.416
12	103497	0.378
16	103141	0.377
20	91143	0.333
24	60709	0.222

Table E5 Glucose produced from the hydrolysis of 60–80 mesh corncob using the bacterial strain M 015 at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	101255	0.370
1	89599	0.327
2	91335	0.334
3	103128	0.377
4	104250	0.381
5	102996	0.376
6	107161	0.391
8	114012	0.416
10	118160	0.432
12	107503	0.393
16	105515	0.385
20	77327	0.282
24	57120	0.209

Table E6 Glucose produced from the hydrolysis of 80–100 mesh corncob using the bacterial strain M 015 at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	111323	0.407
1	90886	0.332
2	107221	0.392
3	110414	0.403
4	109486	0.400
5	112719	0.412
6	120857	0.441
8	125769	0.459
10	106723	0.390
12	107547	0.393
16	100179	0.366
20	81812	0.299
24	61566	0.225

Table E7 Glucose produced from the hydrolysis of 80–100 mesh corncob using malt extract loading 12 g/L and bacterial strain A 002 at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	94051	0.343
1	47470	0.173
2	34637	0.127
3	80681	0.295
4	85262	0.311
5	111717	0.408
6	219508	0.802
8	133258	0.487
10	140588	0.513
12	130418	0.476
14	100965	0.369
16	87803	0.321
20	88868	0.325
24	87304	0.319

Table E8 Glucose produced from the hydrolysis of 80–100 mesh corncob using malt extract loading 10 g/L and bacterial strain A 002 at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	71193	0.260
1	33753	0.123
2	23873	0.087
3	54836	0.200
4	59781	0.218
5	79599	0.291
6	176008	0.643
8	111416	0.407
10	117070	0.428
12	107854	0.394
14	81798	0.299
16	67297	0.246
20	65679	0.240
24	64277	0.235

Table E9 Glucose produced from the hydrolysis of 80–100 mesh corncob using malt extract loading 8 g/L and bacterial strain A 002 at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	54160	0.198
1	28821	0.105
2	23873	0.087
3	46895	0.171
4	61249	0.224
5	78438	0.286
6	131429	0.480
8	109524	0.400
10	99861	0.365
12	84810	0.310
14	67250	0.246
16	60266	0.220
20	55438	0.202
24	55786	0.204

Table E10 Glucose produced from the hydrolysis of 80–100 mesh corncob using malt extract loading 6 g/L and bacterial strain A 002 at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	35656	0.130
1	29571	0.108
2	19397	0.071
3	61336	0.224
4	72198	0.264
5	85453	0.312
6	113823	0.416
8	93005	0.340
10	57032	0.208
12	48366	0.177
14	42361	0.155
16	36189	0.132
20	35560	0.130
24	34429	0.126

Table E11 Glucose produced from the enzymatic hydrolysis of 80–100 mesh corncob using 100 U cellulase enzyme loading at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	0	0.000
2	9663	0.035
4	17532	0.064
6	23427	0.086
8	27696	0.101
10	31836	0.116
12	36053	0.132
24	49603	0.181
48	63779	0.233
72	69871	0.255

Table E12 Glucose produced from the enzymatic hydrolysis of 80–100 mesh corncob using 200 U cellulase enzyme loading at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	0	0.000
2	17674	0.065
4	33150	0.121
6	45734	0.167
8	57207	0.209
10	66476	0.243
12	72190	0.264
24	98287	0.359
48	119173	0.435
72	129841	0.474

Table E13 Glucose produced from the enzymatic hydrolysis of 80–100 mesh corncob using 300 U cellulase enzyme loading at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	0	0.000
2	21119	0.077
4	46466	0.170
6	64069	0.234
8	81787	0.299
10	92047	0.336
12	102441	0.374
24	136878	0.500
48	163300	0.596
72	181197	0.662

Table E14 Glucose produced from the enzymatic hydrolysis of 80–100 mesh corncob using 400 U cellulase enzyme loading at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	0	0.000
2	25201	0.092
4	57103	0.209
6	78230	0.286
8	97170	0.355
10	112736	0.412
12	119159	0.435
24	154018	0.563
48	182054	0.665
72	199410	0.728

Table E15 Glucose produced from the enzymatic hydrolysis of 80–100 mesh corncob using 500 U cellulase enzyme loading at 37 °C.

Time (h)	Glucose	
	Area	Concentration (g/L)
0	0	0.000
2	25527	0.093
4	61123	0.223
6	83309	0.304
8	102514	0.374
10	118133	0.431
12	129134	0.472
24	166222	0.607
48	200445	0.732
72	220759	0.806

Table E16 Bacteria evolution from the hydrolysis of 80–100 mesh corncob with the bacterial strain A 002 at 37 °C.

Time (h)	Nitrogen bacteria (g/L)	Bacterial (g/L)
0	0.062	0.55
3	0.085	0.75
6	0.088	0.78
8	0.087	0.77
10	0.094	0.83
12	0.096	0.85
16	0.111	0.98
20	0.123	1.09
24	0.172	1.53

Table E17 Bacteria evolution from the hydrolysis of 80–100 mesh corncob with the bacterial strain M 015 at 37 °C.

Time (h)	Nitrogen bacteria (g/L)	Bacterial (g/L)
0	0.089	0.79
3	0.090	0.80
6	0.092	0.82
8	0.100	0.89
10	0.102	0.91
12	0.109	0.97
16	0.110	0.98
20	0.112	0.99
24	0.145	1.29

Appendix F SEM images of before and after hydrolysis of corncob

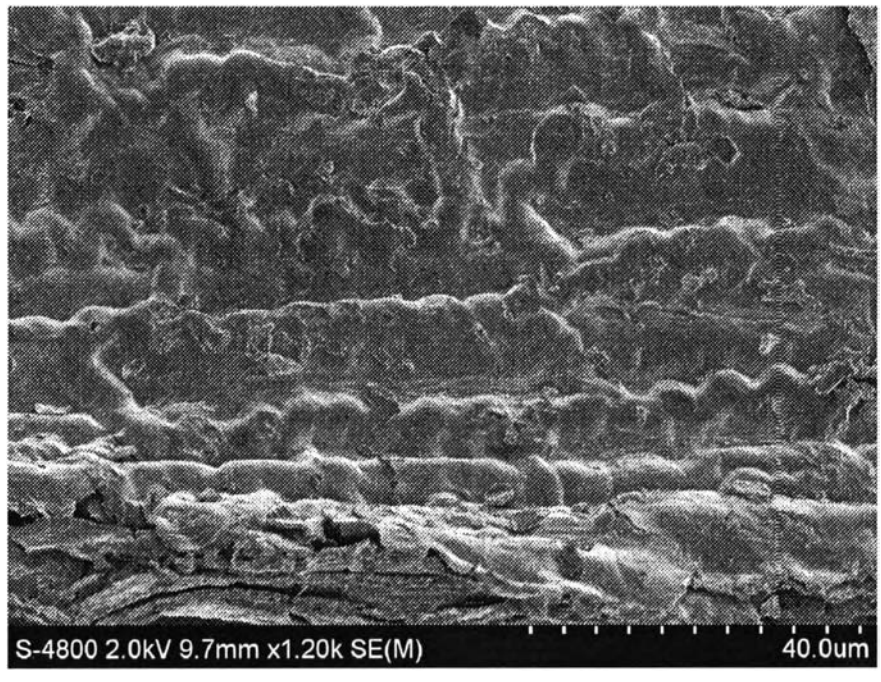

Figure F1 Scanning electron micrographs of the corncob surface before hydrolysis.

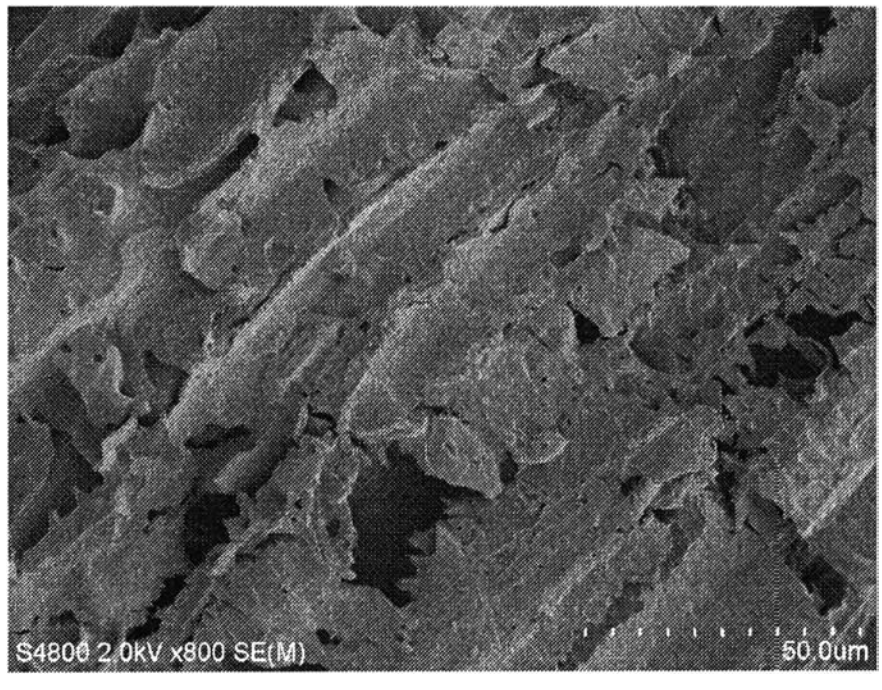

Figure F2 Scanning electron micrographs of the 60–80 mesh corncob surface after hydrolysis using strain A 002 at 37 °C.

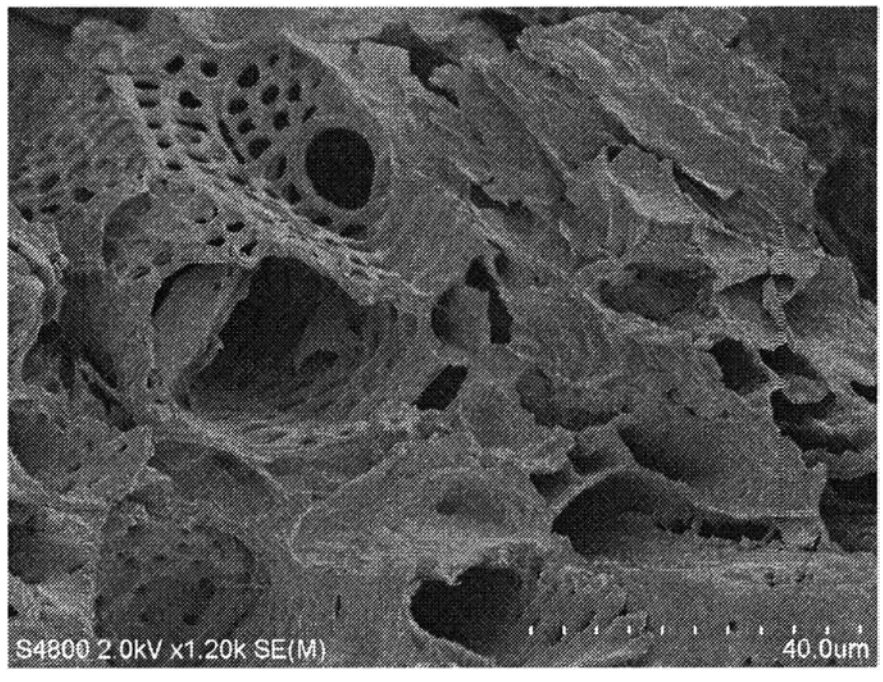

Figure F3 Scanning electron micrographs of the 80–100 mesh corncob surface after hydrolysis using strain A 002 at 37 °C.

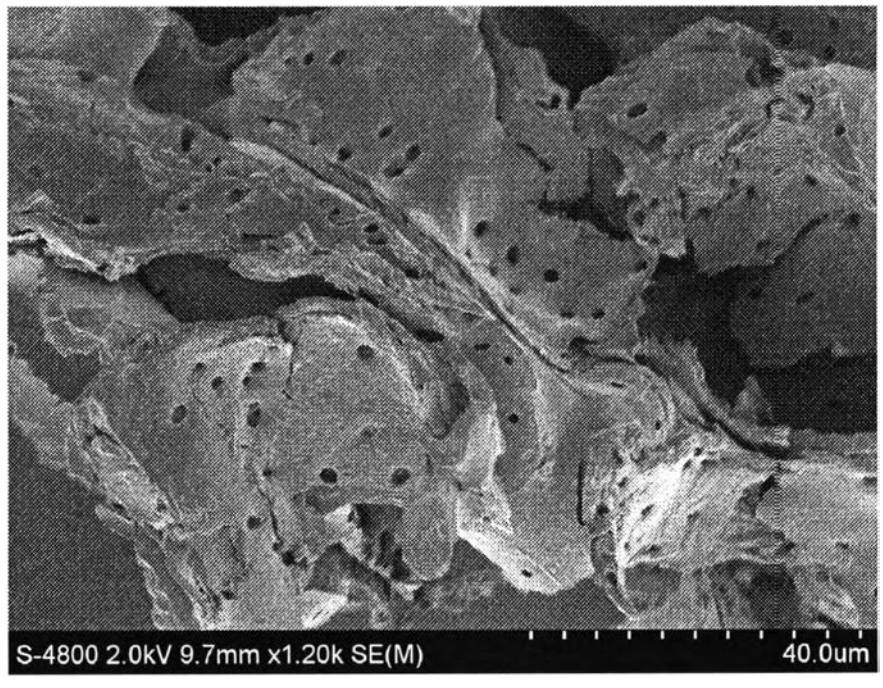

Figure F4 Scanning electron micrographs of the 80–100 mesh corncob surface after hydrolysis using strain M 015 at 37 °C.

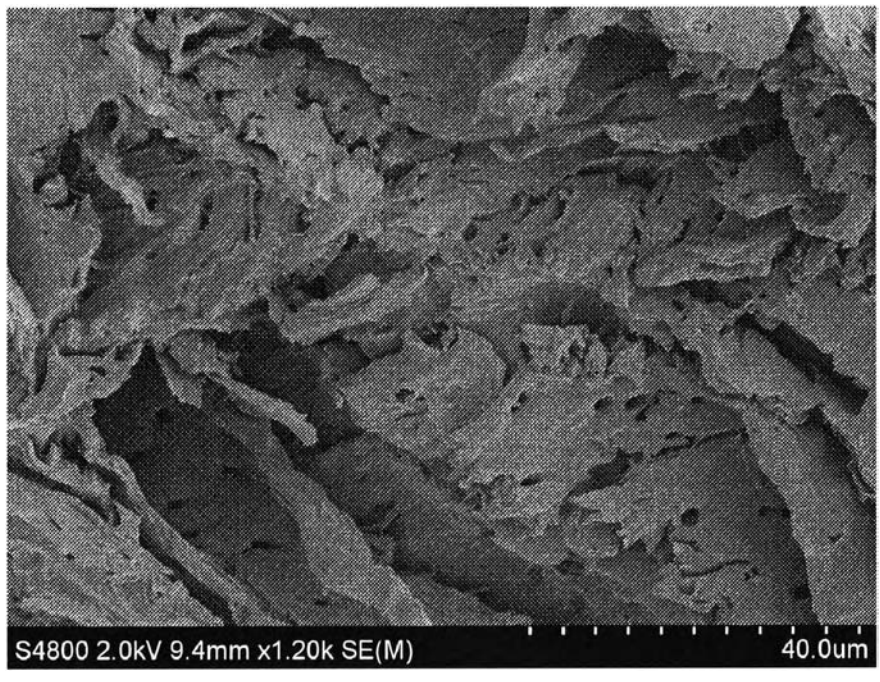

Figure F5 Scanning electron micrographs of the 80–100 mesh corncob surface after enzymatic hydrolysis using 100 U cellulase enzyme loading at 37 °C.

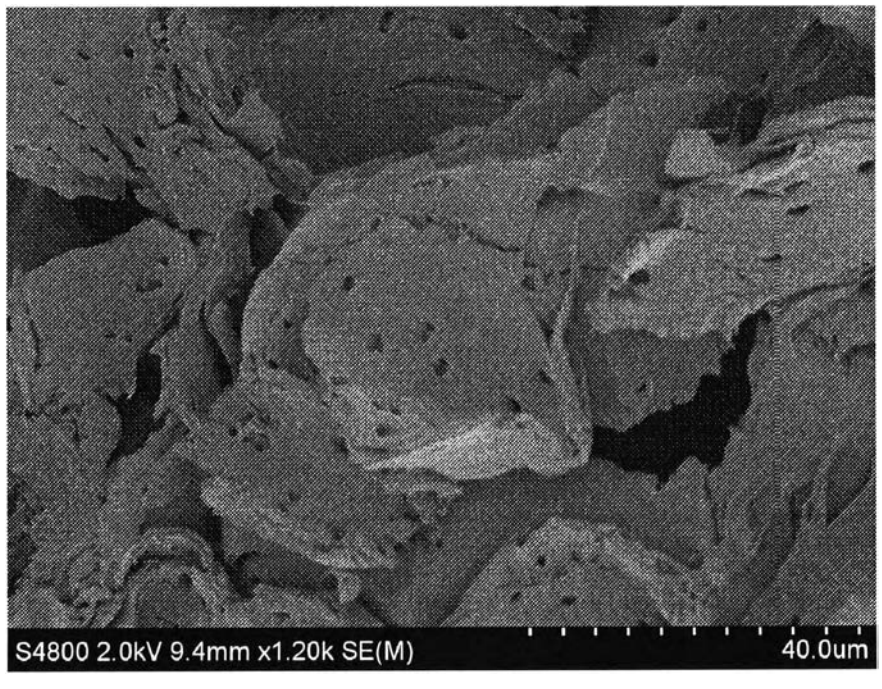

Figure F6 Scanning electron micrographs of the 80–100 mesh corncob surface after enzymatic hydrolysis using 300 U cellulase enzyme loading at 37 °C.

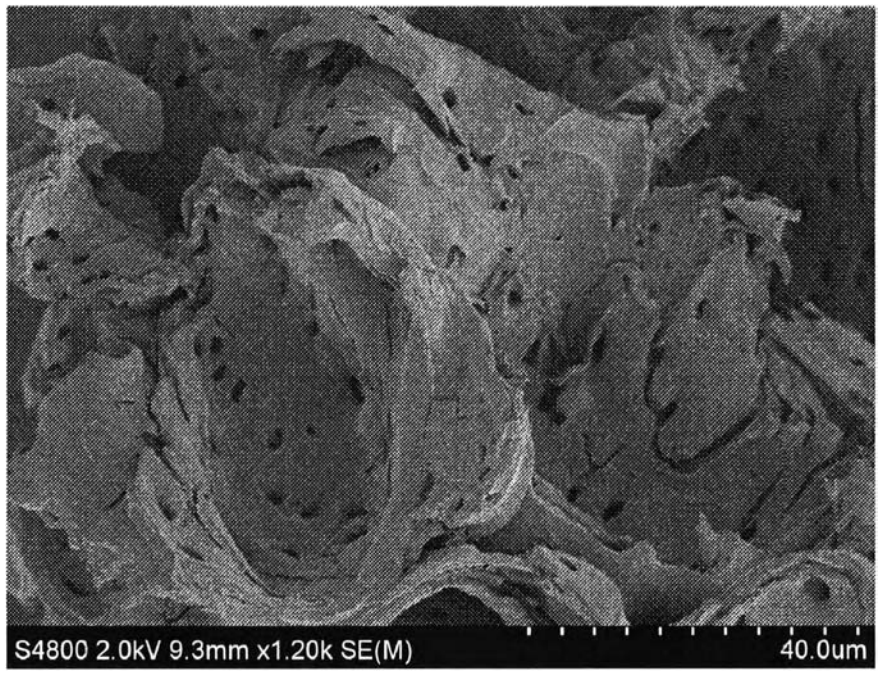

Figure F7 Scanning electron micrographs of the 80–100 mesh corncob surface after enzymatic hydrolysis using 500 U cellulase enzyme loading at 37 °C.

CURRICULUM VITAE

Name: Mr. Nattawut Hokittikul

Date of Birth: August 7, 1988

Nationality: Thai

University Education:

2008-2011 Bachelor Degree of Chemical Engineering, Department of Chemical Technology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

Work Experience:

2010 Position: Process Engineer (Student Internship)
Company: Thai Oleochemicals Co., Ltd

Proceedings:

1. Hokittikul, N., Rangsunvigit, P., and Chavadej, S., (2013, May 12-16) Glucose Production from Corncob by Microbial Hydrolysis. Poster presentation of 2th International Conference on Materials for Energy EnMat II, Karlsruhe, Germany.
2. Hokittikul, N., Rangsunvigit, P., and Chavadej, S., (2013, April 23) Glucose Production from Corncob by Microbial Hydrolysis Using Bacteria Isolated from Thai Higher Termites. Proceedings of The 4th Research Symposium on Petrochemical and Materials Technology and The 19th PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.