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APPENDICES

Appendix A Standard Calibration Curve

1. Glucose Calibration Curve

Table A1 Glucose calibration curve

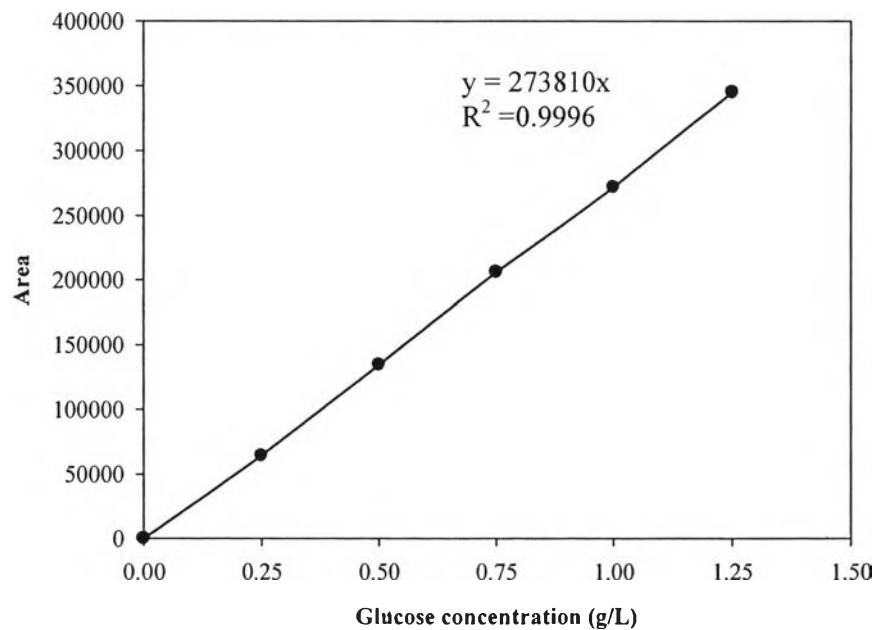| Glucose concentration (g/L)	Area(glucose)
0.00	0
0.25	64231
0.50	134469
0.75	206002
1.00	272024
1.25	345123

$$y = 273810x$$
$$R^2 = 0.9996$$

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

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 N in 100 mL

Hydrochloric acid (HCl conc.)	8.29	mL
Distilled water	91.71	mL

3. Sodium hydroxide 0.5 N in 1000 mL

Sodium hydroxide (NaOH)	5.0	g
Distilled water	1000	mL

4. Sulfuric acid 0.72 N 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
Distilled water	950	mL

6. 0.2 M Acetic acid in 1000 mL

Acetic 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 bagasse and bacteria. Method that can calculate weight of bacteria and bagasse is shown in equation D1.

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

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

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

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 D1 shows procedure for determination
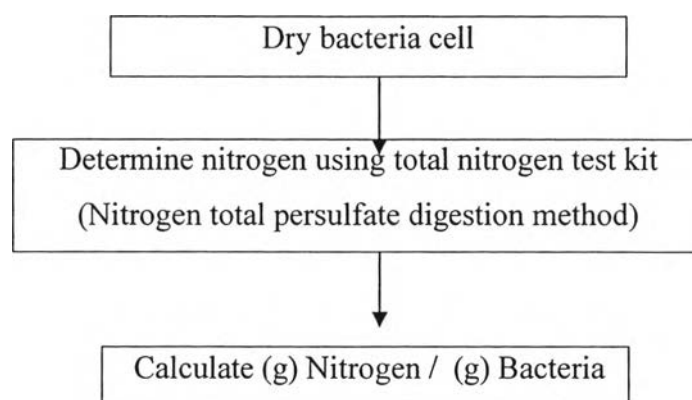

Figure D1 Diagram for determination the amount of nitrogen in bacteria.

Procedure

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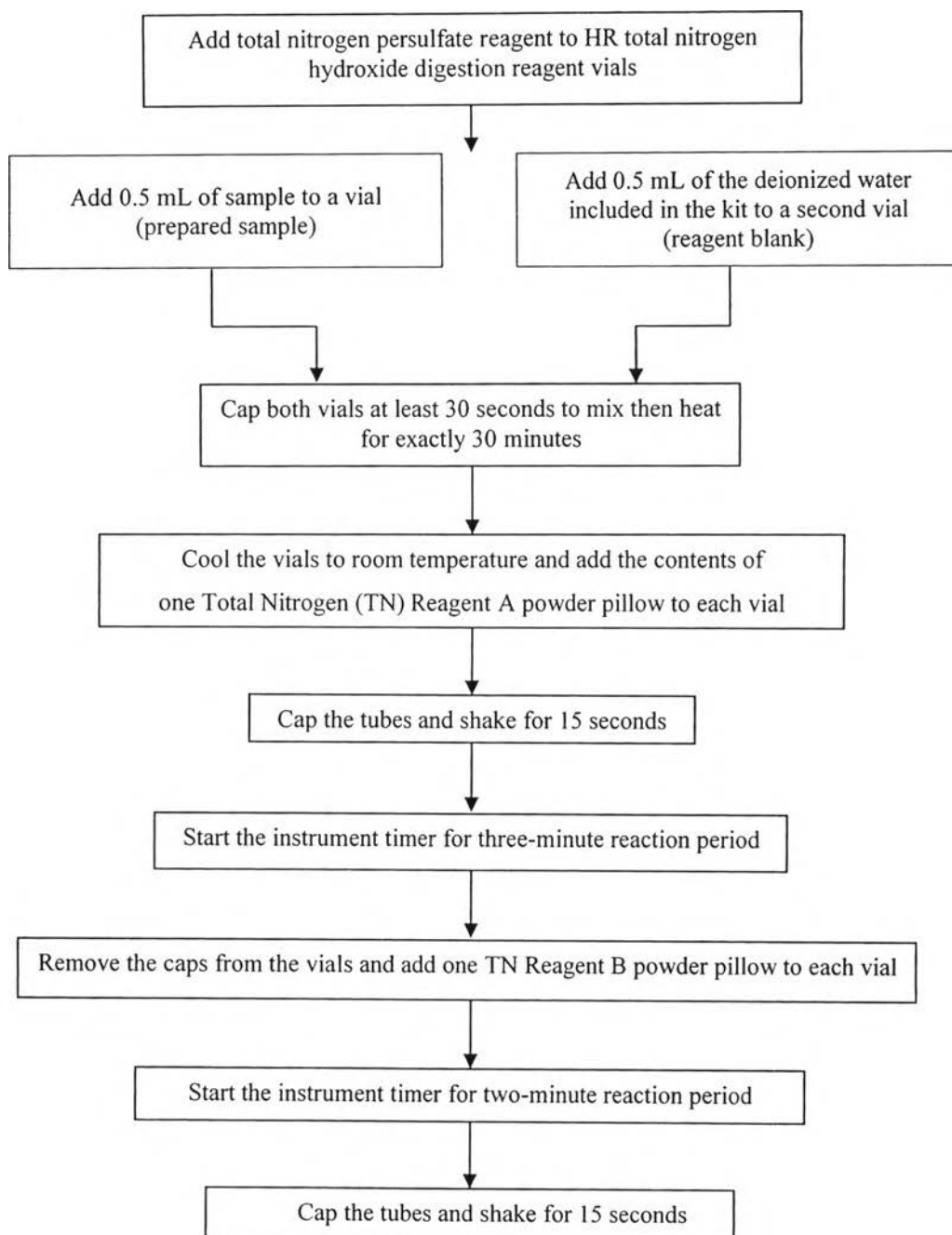

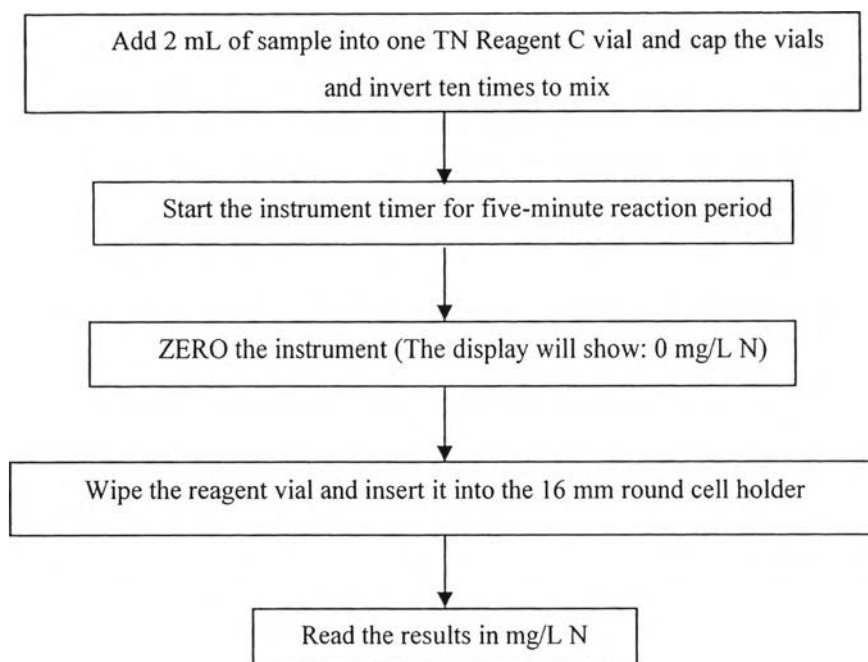**Figure D2** Procedure for analyzing amount of nitrogen.

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

Appendix E Experiment Data of Enzymatic Hydrolysis

Table E1 Glucose produced from the hydrolysis of 40–60 mesh bagasse with strain A 002 at 37 °C

Time (h)	Area	Glucose (g/L)
0	90026	0.329
1	67516	0.247
2	64329	0.235
3	66629	0.243
4	79049	0.289
5	86026	0.314
6	91855	0.335
8	103678	0.379
10	76461	0.279
12	69753	0.255
14	58803	0.215
16	30664	0.112
20	16374	0.060
24	6626	0.024

Table E2 Glucose produced from the hydrolysis of 40–60 mesh bagasse with strain M 015 at 37 °C

Time (h)	Area	Glucose (g/L)
0	107506	0.393
1	94771	0.346
2	98470	0.360
3	99067	0.362
4	97624	0.357
5	84336	0.308
6	92389	0.337
8	101362	0.370
10	102621	0.375
12	106709	0.390
14	106731	0.390
16	109264	0.399
20	82603	0.302
24	84019	0.307

Table E3 Glucose produced from the hydrolysis of 60–80 mesh bagasse with strain A 002 at 37 °C

Time (h)	Area	Glucose (g/L)
0	107043	0.391
1	68168	0.249
2	57407	0.210
3	84903	0.310
4	89185	0.326
5	93188	0.340
6	96852	0.354
8	111890	0.409
10	82384	0.301
12	81135	0.296
14	73701	0.269
16	33175	0.121
20	19884	0.073
24	10136	0.037

Table E4 Glucose produced from the hydrolysis of 60–80 mesh bagasse with strain M 015 at 37 °C

Time (h)	Area	Glucose (g/L)
0	109371	0.399
1	94374	0.345
2	74851	0.273
3	79736	0.291
4	84624	0.309
5	79621	0.291
6	77937	0.285
8	93120	0.340
10	105529	0.385
12	107073	0.391
14	112276	0.410
16	107892	0.394
20	65807	0.240
24	67568	0.247

Table E5 Glucose produced from the hydrolysis of > 80 mesh bagasse with strain A 002 at 37 °C

Time (h)	Area	Glucose (g/L)
0	89117	0.325
1	56947	0.208
2	50452	0.184
3	63127	0.231
4	84355	0.308
5	85919	0.314
6	112224	0.410
8	125725	0.459
10	102985	0.376
12	87512	0.320
14	41422	0.151
16	27211	0.099
20	7919	0.029
24	5180	0.019

Table E6 Glucose produced from the hydrolysis of > 80 mesh bagasse with strain M 015 at 37 °C

Time (h)	Area	Glucose (g/L)
0	96770	0.353
1	81458	0.298
2	75166	0.275
3	86587	0.316
4	86721	0.317
5	85160	0.311
6	75834	0.277
8	73581	0.269
10	117103	0.428
12	120263	0.439
14	110252	0.403
16	99346	0.363
18	88323	0.323
20	71237	0.260
24	96770	0.353

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

Time (h)	Area	Glucose (g/L)
0	96567	0.353
1	91108	0.333
2	59359	0.217
3	9129	0.033
4	15238	0.056
5	64501	0.236
6	84796	0.310
8	125684	0.459
10	113563	0.415
12	92096	0.336
14	82061	0.300
16	88791	0.324
20	75355	0.275
24	54439	0.199

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

Time (h)	Area	Glucose (g/L)
0	80251	0.293
1	53374	0.195
2	27326	0.100
3	26034	0.095
4	33911	0.124
5	65109	0.238
6	86458	0.316
8	127675	0.466
10	108514	0.396
12	98462	0.360
14	71692	0.262
16	65170	0.238
20	40368	0.147
24	35300	0.129

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

Time (h)	Area	Glucose (g/L)
0	63316	0.231
1	28575	0.104
2	13003	0.047
3	18896	0.069
4	23594	0.086
5	67075	0.245
6	86406	0.316
8	107755	0.394
10	91313	0.333
12	76122	0.278
14	38517	0.141
16	28520	0.104
20	24429	0.089
24	21962	0.080

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

Time (h)	Area	Glucose (g/L)
0	38637	0.141
1	15547	0.057
2	29174	0.107
3	26179	0.096
4	32436	0.118
5	68729	0.251
6	80484	0.294
8	99845	0.365
10	81968	0.299
12	21434	0.078
14	13102	0.048
16	9958	0.036
18	9671	0.035
20	10599	0.039
24	10972	0.040

Table E11 Glucose and Bacteria evolution from the microbial hydrolysis of > 80 mesh bagasse with strain A 002 at 37 °C

Time (h)	Area	Glucose Concentration (g/L)	Nitrogen Bacteria (g/L)	Bacteria (g/L)
0	89117	0.325	0.054	0.479
3	63127	0.231	0.078	0.692
6	112224	0.410	0.094	0.834
8	125725	0.459	0.105	0.932
10	102985	0.376	0.110	0.976
12	87512	0.320	0.109	0.967
16	27211	0.099	0.115	1.020
20	7919	0.029	0.114	1.012
24	5180	0.019	0.130	1.154

Table E12 Glucose and Bacteria evolution from the microbial hydrolysis of > 80 mesh bagasse with strain M 015 at 37 °C

Time (h)	Area	Glucose Concentration (g/L)	Nitrogen Bacteria (g/L)	Bacteria (g/L)
0	96770	0.353	0.094	0.834
3	86587	0.316	0.088	0.781
6	75834	0.277	0.088	0.781
8	73581	0.269	0.098	0.870
10	117103	0.428	0.091	0.807
12	120263	0.439	0.093	0.825
16	99346	0.363	0.097	0.861
20	71237	0.323	0.102	0.905
24	96770	0.260	0.117	1.038

Appendix F Experiment Data of Enzymatic Hydrolysis

Table F1 Glucose produced from the enzymatic hydrolysis of > 80 mesh bagasse particle sizes using 100 U cellulase enzyme loading.

Time (h)	Area	Glucose (g/L)
0	38637	0.00000
2	15547	0.01910
4	29174	0.03614
6	26179	0.04426
8	32436	0.05807
10	68729	0.06499
12	80484	0.07441
24	99845	0.11186
48	81968	0.15604
72	21434	0.18973

Table F2 Glucose produced from the enzymatic hydrolysis of > 80 mesh bagasse particle sizes using 200 U cellulase enzyme loading.

Time (h)	Area	Glucose (g/L)
0	0	0.000
2	5624	0.021
4	12119	0.044
6	21119	0.077
8	25779	0.094
10	31255	0.114
12	35201	0.129
24	51427	0.188
48	67089	0.245
72	77223	0.282

Table F3 Glucose produced from the enzymatic hydrolysis of > 80 mesh bagasse particle sizes using 300 U cellulase enzyme loading.

Time (h)	Area	Glucose (g/L)
0	0	0.000
2	5460	0.020
4	17507	0.064
6	27753	0.101
8	34771	0.127
10	43018	0.157
12	48492	0.177
24	68803	0.251
48	89692	0.328
72	106474	0.389

Table F4 Glucose produced from the enzymatic hydrolysis of > 80 mesh bagasse particle sizes using 400 U cellulase enzyme loading.

Time (h)	Area	Glucose (g/L)
0	0	0.000
2	8587	0.031
4	25536	0.093
6	36200	0.132
8	45236	0.165
10	52917	0.193
12	59921	0.219
24	79440	0.290
48	103497	0.378
72	122908	0.449

Table F5 Glucose produced from the enzymatic hydrolysis of > 80 mesh bagasse particle sizes using 500 U cellulase enzyme loading.

Time (h)	Area	Glucose (g/L)
0	0	0.000
2	9991	0.036
4	27937	0.102
6	40015	0.146
8	48834	0.178
10	55025	0.201
12	64082	0.234
24	87378	0.319
48	119507	0.436
72	140659	0.514

Appendix G SEM images of before and after enzymatic hydrolysis of bagasse
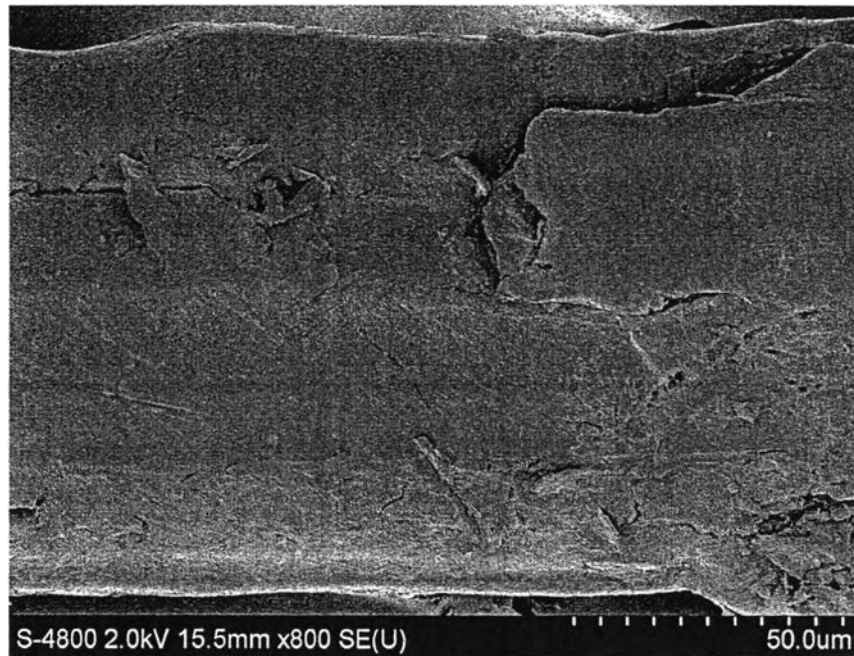
Figure G1 Scanning electron micrographs of the bagasse surface before hydrolysis of > 80 mesh bagasse.
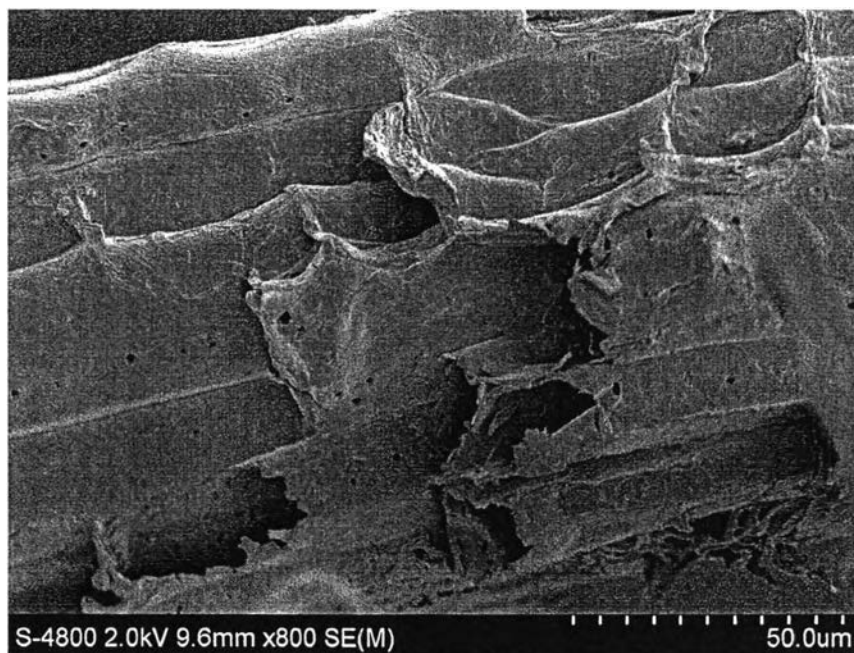
Figure G2 Scanning electron micrographs of the bagasse surface after hydrolysis of > 80 mesh bagasse with strain A 002 at 37 °C.
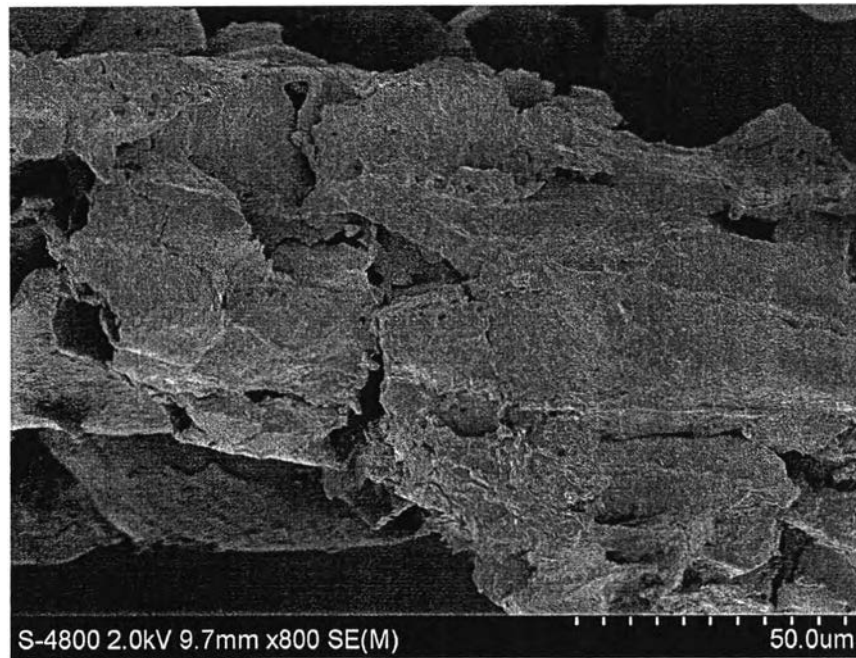
Figure G3 Scanning electron micrographs of the bagasse surface after hydrolysis of > 80 mesh bagasse with strain M 015 at 37 °C.
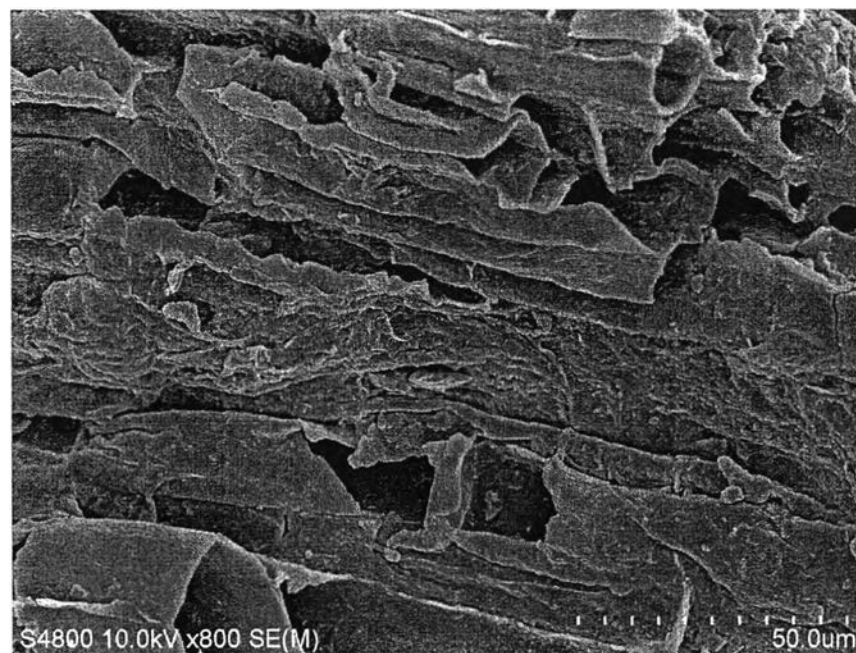

Figure G4 Scanning electron micrographs of the bagasse surface after hydrolysis of 60–80 mesh bagasse with strain A 002 at 37 °C.
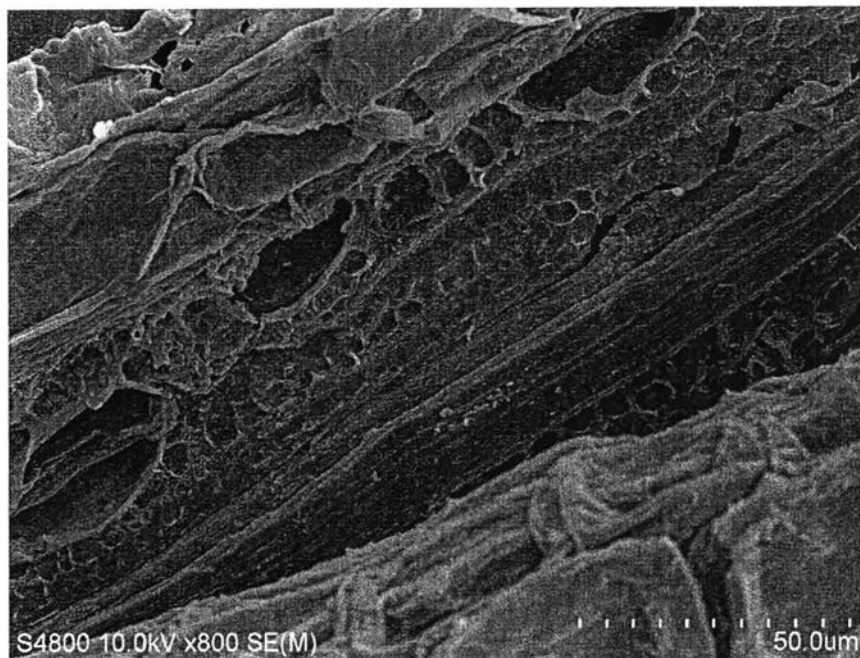
Figure G5 Scanning electron micrographs of the bagasse surface after hydrolysis of 40–60 mesh bagasse with strain A 002 at 37 °C.

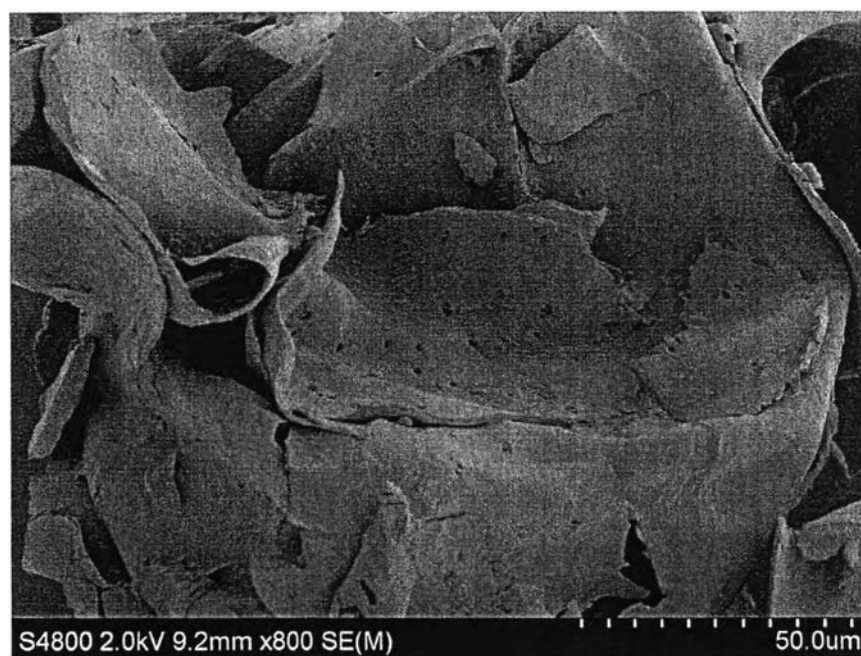**Figure G6** Scanning electron micrographs of the bagasse surface after hydrolysis of > 80 mesh bagasse with 500 U cellulase enzyme loading at 37 °C.

Figure G7 Scanning electron micrographs of the bagasse surface after hydrolysis of > 80 mesh bagasse with 300 U cellulase enzyme loading at 37 °C.
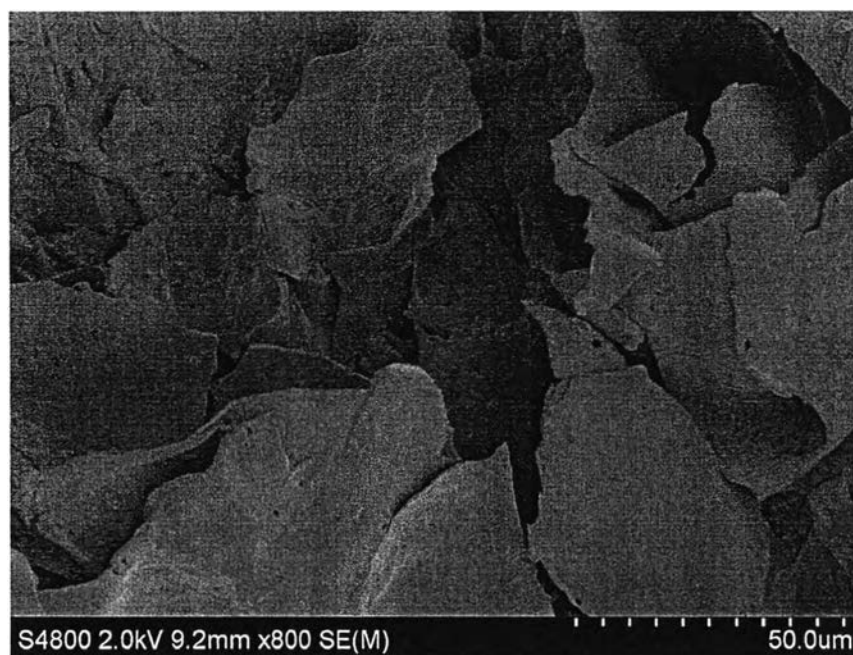

Figure G8 Scanning electron micrographs of the bagasse surface after hydrolysis of > 80 mesh bagasse with 300 U cellulase enzyme loading at 37 °C.

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1. Visuttiewin, S., Chavadej, S., and Rangsunvigit, P. (2013, May 12-16). Conversion of Sugarcane Bagasse to Sugars by Microbial Hydrolysis. Poster presentation of 2th International Conference on Materils for Energy EnMat II, Karlruhe, Germany.

2. Visuttiewin, S., Chavadej, S., and Rangsunvigit, P. (2013, April 23). Conversion of Sugarcane Bagasse to Sugars by Microbial Hydrolysis Using Bacteria Isolated from Thai Higher Termites. Proceedings of the 4th Research Symposium on Petrochemical and Materials Technology and 19th PPC Symposium on Petroleum, Petrochemical, and Polymers, Bangkok, Thailand.