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APPENDICES

Appendix A Standard Glucose Calibration Curve

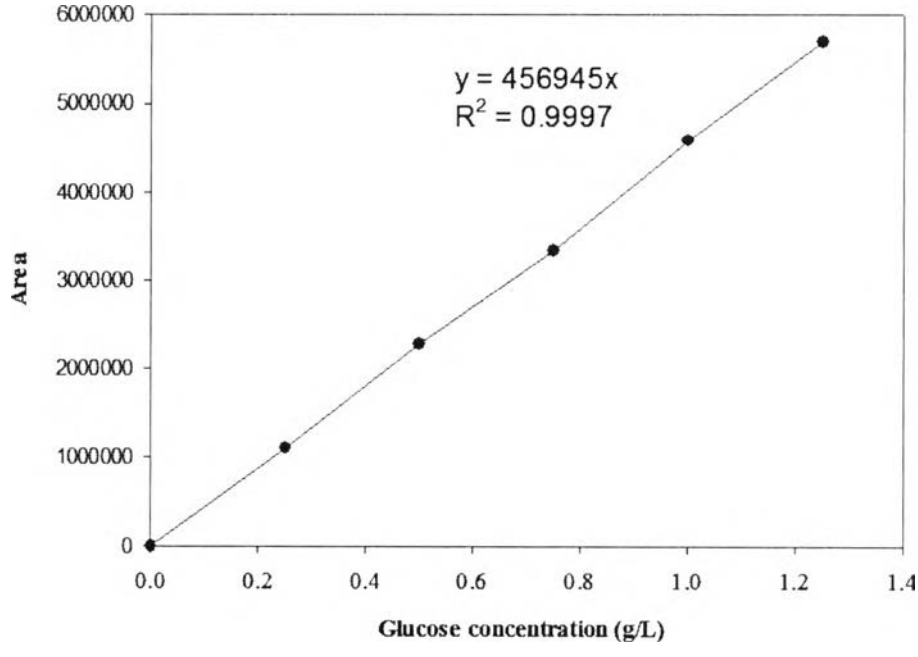

Figure A1 Calibration curve for sugar analysis HPLC glucose determination.

Table A1 Glucose calibration curve

Glucose Concentration (g/L)	Avg.Glucose Area
0	0
0.25	110731
0.50	228073
0.75	333867
1.00	458414
1.25	569885.5

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 Temperature = 121°C and Pressure = 15 lbs/in^2 for 20 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 Temperature = 121°C and Pressure = 15 lbs/in^2 for 20 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. Sulfuric acid (0.9 %w/w)

Sulfuric acid (H_2SO_4 conc.)	0.9	g
Distilled water	100	mL

6. 0.2 M Acetic acid in 1000 mL

Acetic acid (CH_3COOH)	11.6	mL
Distilled water	988.4	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.

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

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 D1 shows procedure for determination

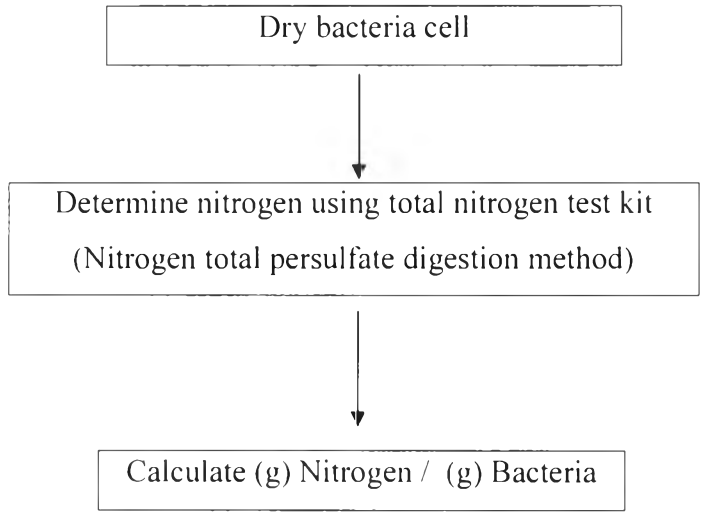

Figure D1 Diagram for determination the amount of nitrogen in bacteria.

Procedure

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

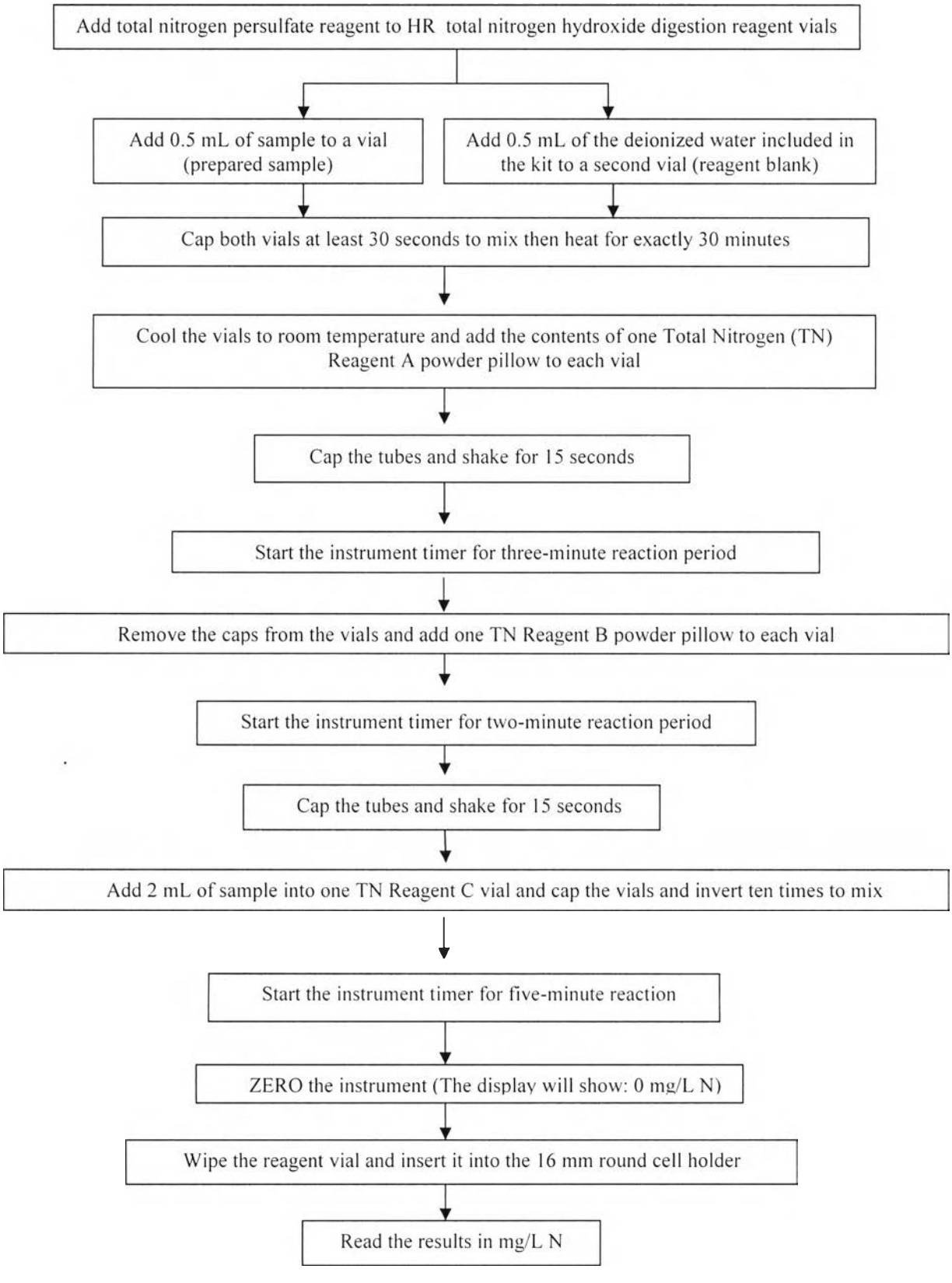

Figure D2 Procedure for analyzing amount of nitrogen.

Appendix E HPLC Analysis

Table E1 Glucose production from the microbial hydrolysis of untreated corncob (80 mesh) with bacteria strain A002 at 37 °C.

Time (h)	Area	Glucose concentration (g/L)
0	93389	0.204
1	66076	0.145
2	49994	0.109
3	91461	0.200
4	90563	0.198
5	120238	0.263
6	121146	0.265
7	96766	0.212
8	94886	0.208
9	107323	0.235
10	81039	0.177
12	54673	0.120
14	21527	0.047
16	10612	0.023
18	8062	0.018
20	4213	0.009
22	4039	0.009
24	5109	0.011

Table E2 Glucose production from the microbial hydrolysis of untreated sugarcane bagasse (80 mesh) with bacteria strain A002 at 37 °C.

Time (h)	Area	Glucose concentration (g/L)
0	17882	0.039
1	16681	0.037
2	20828	0.046
3	40933	0.090
4	60869	0.133
5	77959	0.171
6	54531	0.119
7	42157	0.092
8	48914	0.107
9	46828	0.102
10	30118	0.066
12	18645	0.041
14	8904	0.019
16	7350	0.016
18	6346	0.014
20	7378	0.016
22	7783	0.017
24	10953	0.024

Table E3 Glucose production from the microbial hydrolysis of 1 hour steam-explosion pretreated corncob and sugarcane bagasse by using H_2O as a preimpregnation agent (solid/liquid ratio: 1/10) with bacteria strain A002 at 37 °C.

Time (h)	Glucose concentration from corncob (g/L)	Glucose concentration from Sugarcane bagasse (g/L)
0	0.112	0.055
1	0.048	0.029
2	0.085	0.086
3	0.144	0.142
4	0.236	0.278
5	0.248	0.352
6	0.180	0.369
7	0.140	0.327
8	0.061	0.300
9	0.071	0.250
10	0.145	0.182
12	0.176	0.105
14	0.176	0.018
16	0.086	0.036
18	0.063	0.030
20	0.041	0.074
22	0.024	0.026
24	0.037	0.032

Table E4 Glucose production from the microbial hydrolysis of 2 hour steam-explosion pretreated corncob and sugarcane by using H_2O as a preimpregnation agent (solid/liquid ratio: 1/10) with bacteria strain A002 at 37 °C.

Time (h)	Glucose concentration from corncob (g/L)	Glucose concentration from Sugarcane bagasse (g/L)
0	0.033	0.171
1	0.051	0.105
2	0.089	0.096
3	0.149	0.170
4	0.217	0.266
5	0.268	0.338
6	0.190	0.364
7	0.149	0.237
8	0.141	0.241
9	0.039	0.084
10	0.036	0.035
12	0.025	0.020
14	0.029	0.007
16	0.012	0.005
18	0.007	0.011
20	0.009	0.004
22	0.008	0.009
24	0.008	0.008

Table E5 Glucose production from the microbial hydrolysis of 3 hour steam-explosion pretreated corncob and sugarcane bagasse by using H_2O as a preimpregnation agent (solid/liquid ratio: 1/10) with bacteria strain A002 at 37 °C.

Time (h)	Glucose concentration from corncob (g/L)	Glucose concentration from Sugarcane bagasse (g/L)
0	0.172	0.087
1	0.156	0.051
2	0.100	0.099
3	0.141	0.122
4	0.201	0.206
5	0.259	0.260
6	0.245	0.269
7	0.228	0.236
8	0.151	0.139
9	0.073	0.066
10	0.036	0.035
12	0.019	0.039
14	0.018	0.046
16	0.021	0.005
18	0.018	0.011
20	0.012	0.010
22	0.024	0.008
24	0.030	0.008

Table E6 Glucose production from the microbial hydrolysis of 1 hour steam-explosion pretreated corncob and sugarcane by using H_2SO_4 as a preimpregnation agent (H_2SO_4 0.9% w/w) with bacteria strain A002 at 37 °C.

Time (h)	Glucose concentration from corncob (g/L)	Glucose concentration from Sugarcane bagasse (g/L)
0	0.077	0.201
1	0.062	0.034
2	0.065	0.567
3	0.166	0.600
4	0.249	0.419
5	0.265	0.322
6	0.216	0.292
7	0.137	0.250
8	0.067	0.166
9	0.057	0.126
10	0.033	0.066
12	0.019	0.052
14	0.004	0.046
16	0.007	0.025
18	0.007	0.011
20	0.004	0.009
22	0.008	0.013
24	0.008	0.002

Table E7 Glucose production from the microbial hydrolysis of 2 hour steam-explosion pretreated corncob and sugarcane bagasse by using H_2SO_4 as a preimpregnation agent (H_2SO_4 0.9% w/w) with bacteria strain A002 at 37 °C.

Time (h)	Glucose concentration from corncob (g/L)	Glucose concentration from Sugarcane bagasse (g/L)
0	0.068	0.024
1	0.059	0.056
2	0.084	0.078
3	0.130	0.158
4	0.208	0.488
5	0.246	0.291
6	0.181	0.223
7	0.137	0.156
8	0.101	0.073
9	0.053	0.027
10	0.033	0.034
12	0.015	0.016
14	0.004	0.004
16	0.016	0.005
18	0.008	0.016
20	0.009	0.004
22	0.013	0.009
24	0.002	0.006

Table E8 Glucose production from the microbial hydrolysis of 3 hour steam-explosion pretreated corncob and sugarcane bagasse by using H_2SO_4 as a preimpregnation agent (H_2SO_4 0.9% w/w) with bacteria strain A002 at 37 °C.

Time (h)	Glucose concentration from corncob (g/L)	Glucose concentration from Sugarcane bagasse (g/L)
0	0.022	0.025
1	0.043	0.048
2	0.062	0.089
3	0.173	0.169
4	0.252	0.390
5	0.224	0.542
6	0.180	0.527
7	0.123	0.462
8	0.114	0.287
9	0.071	0.182
10	0.031	0.034
12	0.015	0.040
14	0.003	0.004
16	0.020	0.025
18	0.028	0.041
20	0.023	0.041
22	0.018	0.025
24	0.026	0.035

Appendix F Appearance of the Steam-Explosion Pretreated Corncob and Sugarcane Bagasse

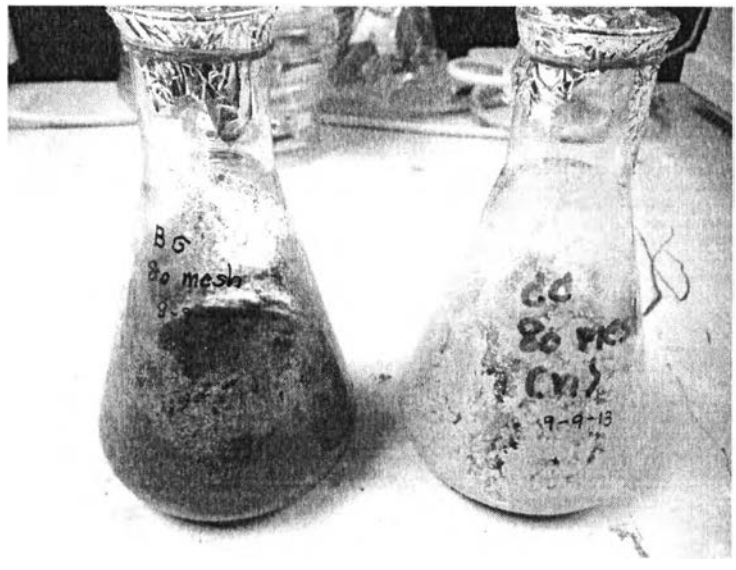

Figure F1 Soaked corncob (right) and bagasse (left) in water (SLR = 1:10) for 24 hours at 60 °C.

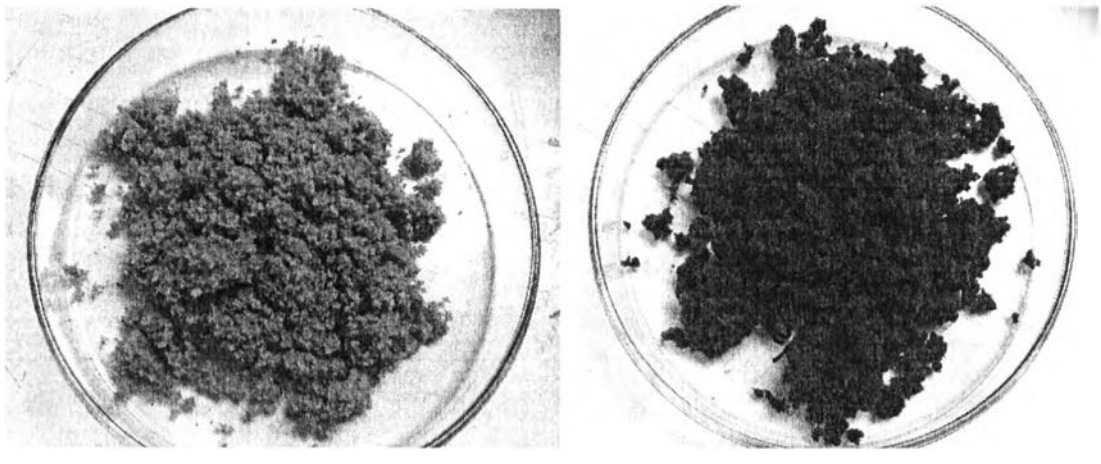

Figure F2 Steam-explosion pretreatment of 80 mesh corncob (left) and bagasse (right) at 120 °C.

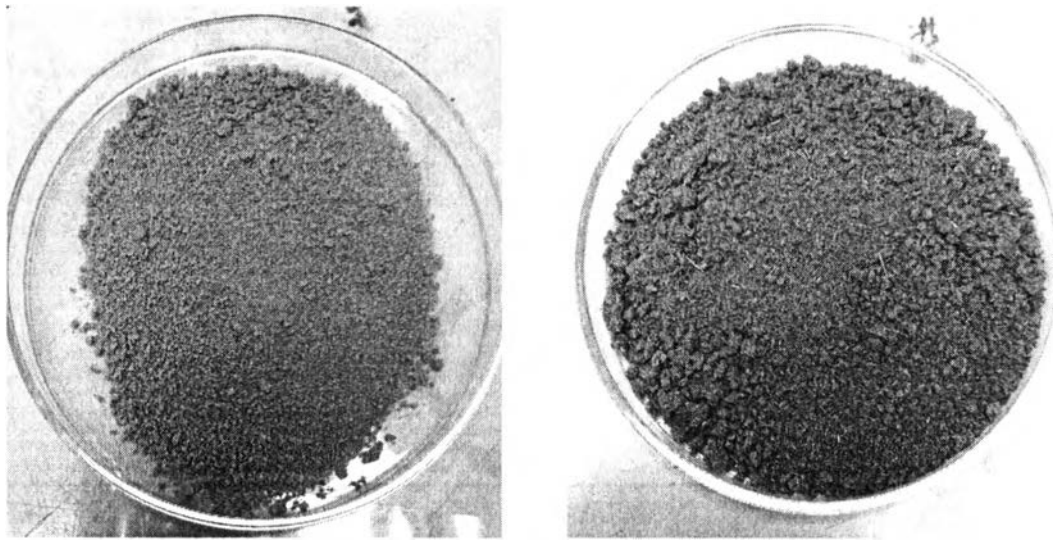

Figure F3 The water-insoluble-fraction (WIS) of corncob (left) and sugarcane bagasse (right) after steam explosion pretreatment and dried at 60 °C overnight for storage.

Appendix G Glucose and Bacteria Evolution Data

Table G1 Glucose and Bacteria evolution from the microbial hydrolysis of untreated sugarcane bagasse with strain A 002 at 37 °C

Time (h)	Glucose Concentration (g/L)	Nitrogen Bacteria (g/L)
0	0.309	0.154
2	0.046	0.278
4	0.133	0.274
6	0.119	0.340
8	0.107	0.479
10	0.066	0.692
12	0.041	0.834
14	0.019	0.932
16	0.016	0.976
18	0.014	0.967
20	0.016	1.020
22	0.017	1.012
24	0.024	1.154

Table G2 Glucose and Bacteria evolution from the microbial hydrolysis of untreated corncob with strain A 002 at 37 °C

Time (h)	Glucose Concentration (g/L)	Nitrogen Bacteria (g/L)
0	0.204	0.115
2	0.109	0.358
4	0.198	0.421
6	0.265	0.468
8	0.208	0.552
10	0.177	0.748
12	0.120	0.780
14	0.047	0.776
16	0.023	0.830
18	0.018	0.850
20	0.009	0.980
22	0.009	1.092
24	0.011	1.211

Table G3 Glucose and Bacteria evolution from the microbial hydrolysis of 2 hour steam-explosion pretreated corncob by using H_2O as a preimpregnation agent with strain A 002 at 37 °C

Time (h)	Glucose Concentration (g/L)	Nitrogen Bacteria (g/L)
0	0.033	0.109
2	0.089	0.313
4	0.217	0.406
6	0.190	0.410
8	0.141	0.427
10	0.036	0.428
12	0.025	0.458
14	0.029	0.459
16	0.012	0.509
18	0.007	0.583
20	0.009	0.593
22	0.008	0.595
24	0.008	0.619

Table G4 Glucose and Bacteria evolution from the microbial hydrolysis of 1 hour steam-explosion pretreated sugarcane bagasse by using H_2O as a preimpregnation agent with strain A 002 at 37 °C

Time (h)	Glucose Concentration (g/L)	Nitrogen Bacteria (g/L)
0	0.055	0.190
2	0.086	0.284
4	0.278	0.294
6	0.369	0.319
8	0.300	0.366
10	0.182	0.392
12	0.105	0.401
14	0.018	0.410
16	0.036	0.420
18	0.030	0.422
20	0.074	0.434
22	0.026	0.469
24	0.032	0.567

Table G5 Glucose and Bacteria evolution from the microbial hydrolysis of 1 hour steam-explosion pretreated corncob by using H_2SO_4 as a preimpregnation agent with strain A 002 at 37 °C

Time (h)	Glucose Concentration (g/L)	Nitrogen Bacteria (g/L)
0	0.077	0.247
2	0.065	0.329
4	0.249	0.393
6	0.216	0.455
8	0.067	0.505
10	0.033	0.537
12	0.019	0.542
14	0.004	0.544
16	0.007	0.590
18	0.007	0.601
20	0.004	0.602
22	0.008	0.631
24	0.008	0.685

Table G6 Glucose and Bacteria evolution from the microbial hydrolysis of 1 hour steam-explosion pretreated sugarcane bagasse by using H_2SO_4 as a preimpregnation agent with strain A 002 at 37 °C

Time (h)	Glucose Concentration (g/L)	Nitrogen Bacteria (g/L)
0	0.201	0.545
2	0.567	0.598
4	0.419	0.647
6	0.292	0.648
8	0.166	0.652
10	0.066	0.679
12	0.052	0.657
14	0.046	0.666
16	0.025	0.673
18	0.011	0.678
20	0.009	0.681
22	0.013	0.686
24	0.002	0.688

Appendix H SEM images of Before and After Microbial Hydrolysis of Corncob and Sugarcane Bagasse

Figure H1 Scanning electron micrographs of the untreated corncob surface before hydrolysis.

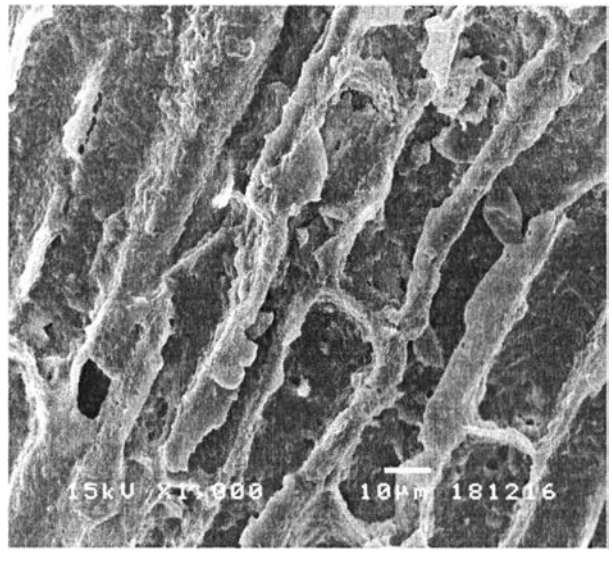

Figure H2 Scanning electron micrographs of the untreated corncob surface after hydrolysis by using bacteria strain A002 at 37 °C.

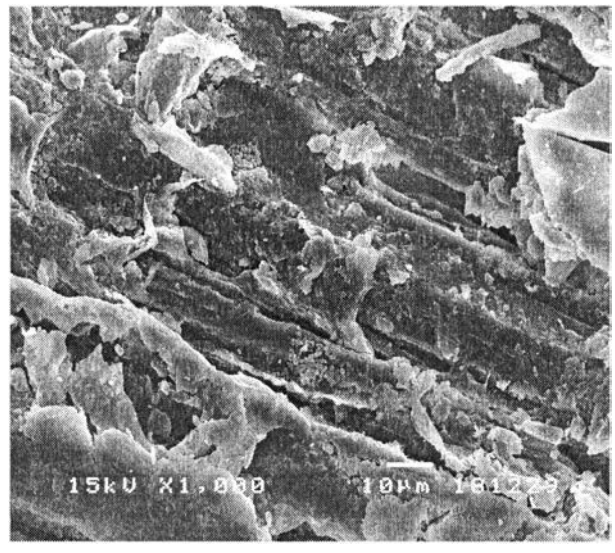

Figure H3 Scanning electron micrographs of the untreated sugarcane bagasse surface before hydrolysis.

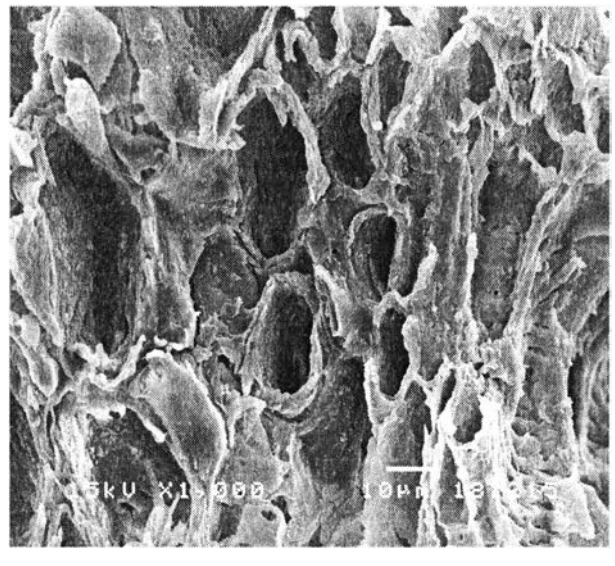

Figure H4 Scanning electron micrographs of the untreated sugarcane bagasse surface after hydrolysis by using bacteria strain A002 at 37 ˚C.

Appendix I SEM images After Steam-Explosion Pretreatment of Corncob and Sugarcane Bagasse

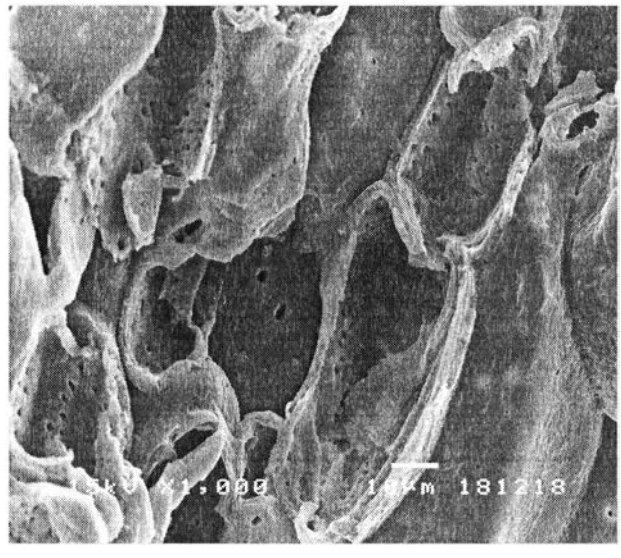

Figure I1 Scanning electron micrographs of the corncob surface after steam-explosion pretreatment with H_2O.

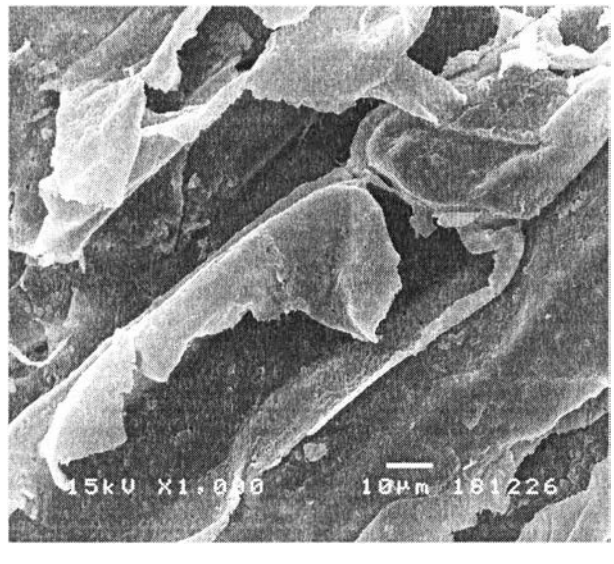

Figure I2 Scanning electron micrographs of the sugarcane bagasse surface after steam-explosion pretreatment with H_2O.

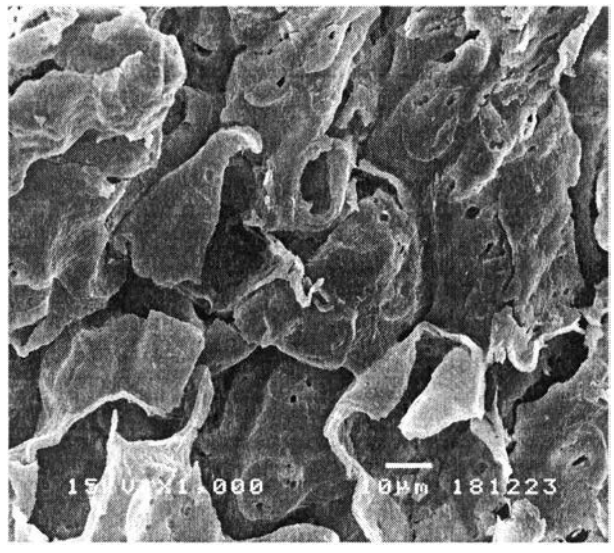

Figure I3 Scanning electron micrographs of the corncob surface after steam-explosion pretreatment with H_2SO_4.

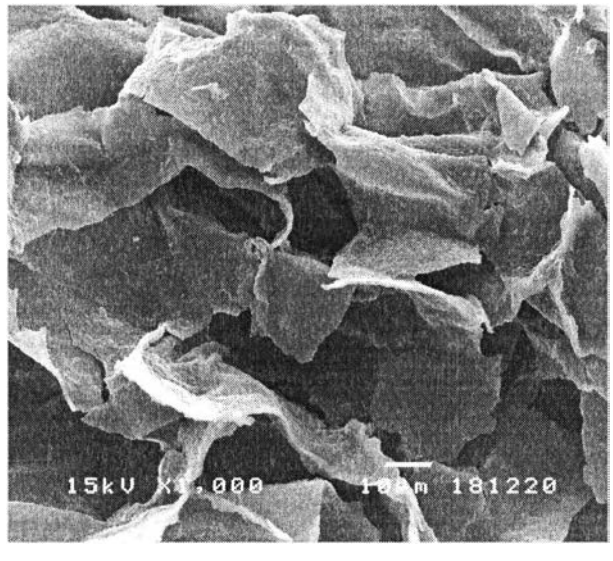

Figure I4 Scanning electron micrographs of the sugarcane bagasse surface after steam-explosion pretreatment with H_2SO_4.

Appendix J Digestibility Calculation of The Chemical Composition Changes

The digestibility of the corncob and sugarcane bagasse residue due to the steam-explosion pretreatment process was determined by performing mass balance, as expressed below:

$$\text{Degradability (\%)} = \frac{\text{Input - Output}}{\text{Input}} \times 100 \qquad \text{(J1)}$$

Table J1 Chemical composition of steam-explosion pretreated corncob and sugarcane bagasse by using H_2O as a preimpregnation agent.

Pretreatment Time (h)	Chemical composition (g)							
	Corncob				Sugarcane bagasse			
	Cellulose	Hemicellulose	Lignin	Extractives	Cellulose	Hemicellulose	Lignin	Extractives
Untreated	0.435	0.320	0.183	0.067	0.429	0.266	0.127	0.197
1	0.289	0.168	0.165	0.059	0.352	0.102	0.115	0.164
2	0.294	0.161	0.173	0.058	0.372	0.0612	0.116	0.171
3	0.268	0.187	0.170	0.057	0.349	0.053	0.106	0.140

Table J2 Chemical composition of steam-explosion pretreated corncob and sugarcane bagasse by using H_2SO_4 as a preimpregnation agent.

Pretreatment Time (h)	Chemical composition (g)							
	Corncob				Sugarcane bagasse			
	Cellulose	Hemicellulose	Lignin	Extractives	Cellulose	Hemicellulose	Lignin	Extractives
1	0.254	0.167	0.170	0.057	0.389	0.062	0.115	0.172
2	0.244	0.167	0.168	0.055	0.380	0.035	0.115	0.163
3	0.241	0.181	0.167	0.055	0.377	0.021	0.114	0.168

Table J3 Degradability from the steam-explosion pretreatment of corncob and sugarcane bagasse by using H_2O as a preimpregnation agent.

Pretreatment Time (h)	Degradability (%)							
	Corncob				Sugarcane bagasse			
	Cellulose	Hemicellulose	Lignin	Extractives	Cellulose	Hemicellulose	Lignin	Extractives
1	33.664	47.490	9.968	9.805	17.967	61.536	9.999	16.743
2	32.515	49.710	5.764	11.633	13.310	76.854	8.977	13.028
3	38.514	41.516	7.020	13.766	18.828	79.940	7.162	28.651

Table J4 Degradability from the steam-explosion pretreatment of corncob and sugarcane bagasse by using H_2SO_4 as a preimpregnation agent.

Pretreatment Time (h)	Degradability (%)							
	Corncob				Sugarcane bagasse			
	Cellulose	Hemicellulose	Lignin	Extractives	Cellulose	Hemicellulose	Lignin	Extractives
1	41.640	47.928	7.456	3.378	9.328	76.854	9.370	12.723
2	43.962	47.709	8.548	4.655	11.424	86.677	9.528	17.303
3	44.697	43.299	8.985	3.004	12.309	91.983	10.392	14.453

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1. Traichedtakul, P., Rangsunvigit, P., and Chavadej, S. (2014, April). Sugar Evolution from Microbial Hydrolysis of Corncob and Sugarcane bagasse: Effect of Pretreatment Steam-Explosion. Proceedings of the 5th Research Symposium on Petrochemical and Materials Technology and 20th PPC Symposium on Petroleum, Petrochemical, and Polymers, Bangkok, Thailand.