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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	0
0.125	1255.945
0.25	2672.915
0.5	6481.374
1.0	12930.928
1.5	19197.000

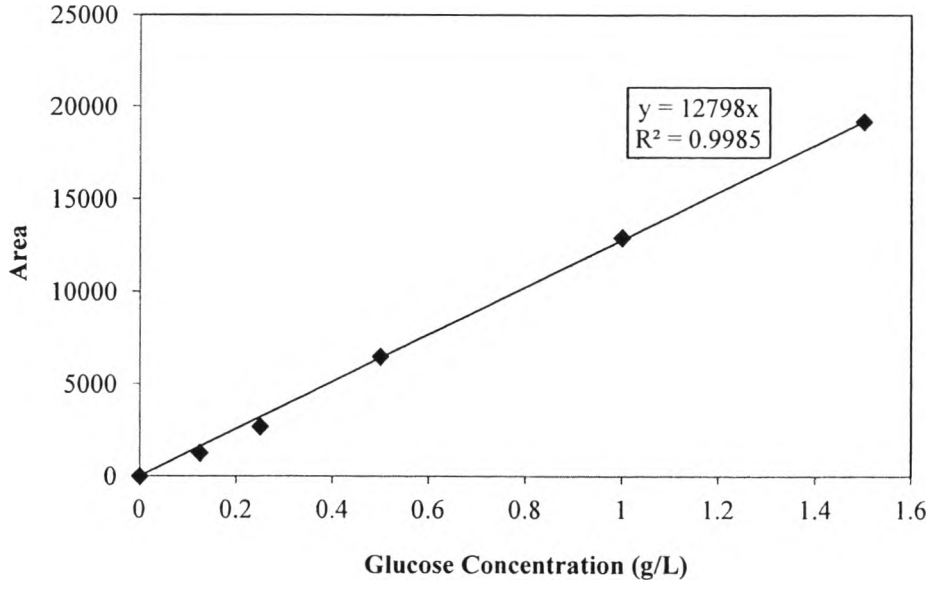

Figure A1 The relationship between glucose concentration (g/L) and area.

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

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

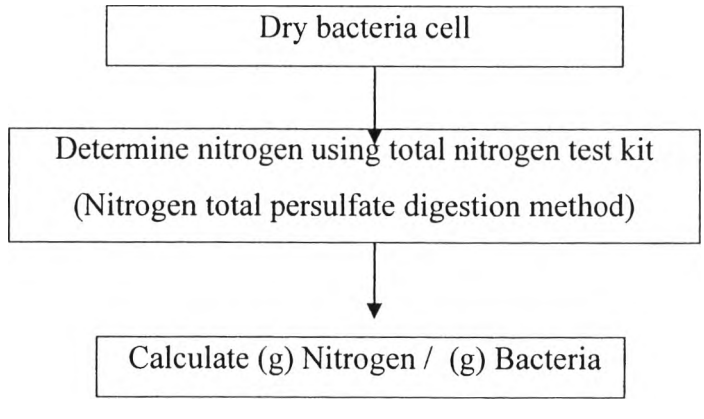

Figure D1 Diagram for determination the amount of nitrogen in bacteria.

Procedure

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

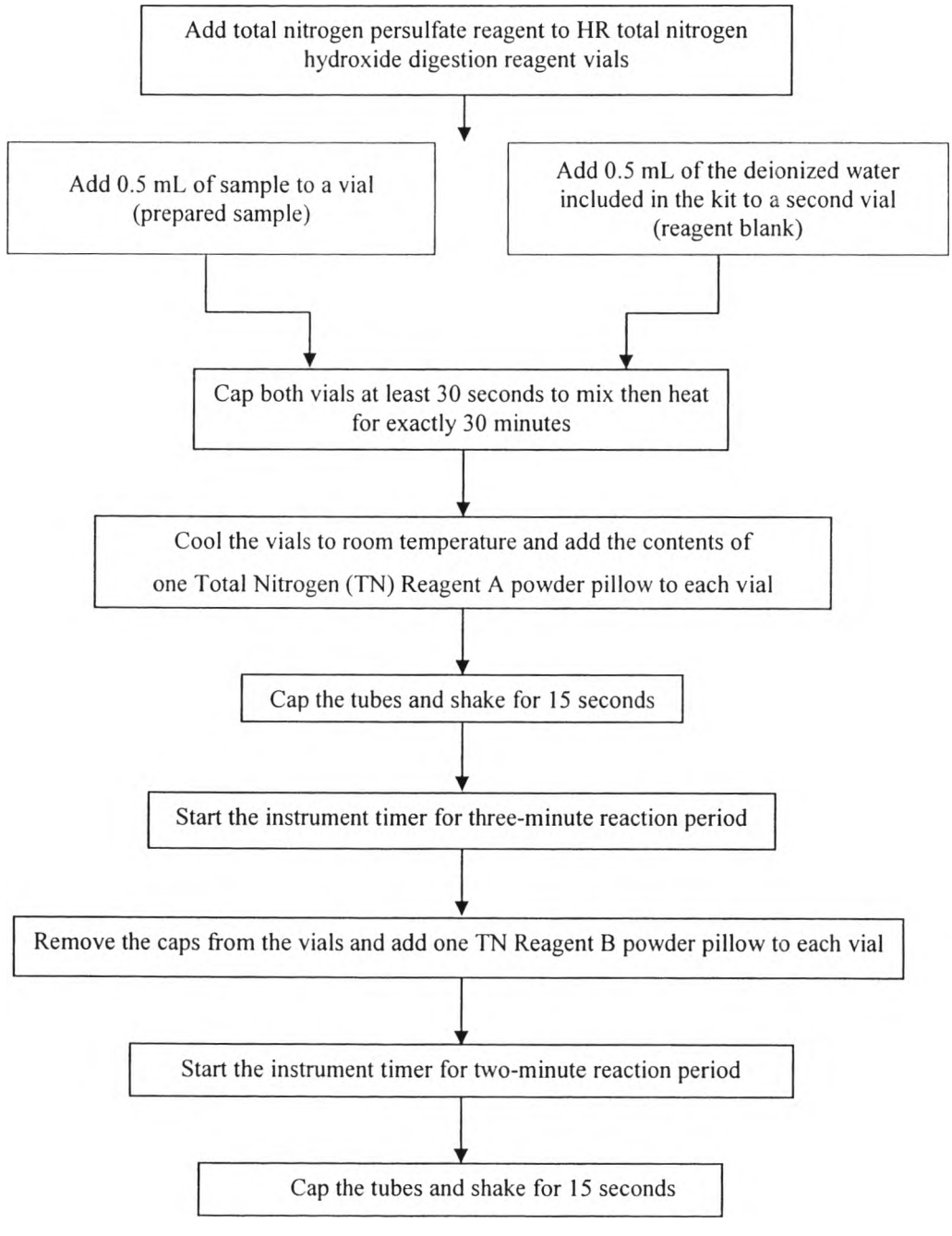

Figure D2 Procedure for analyzing amount of nitrogen.

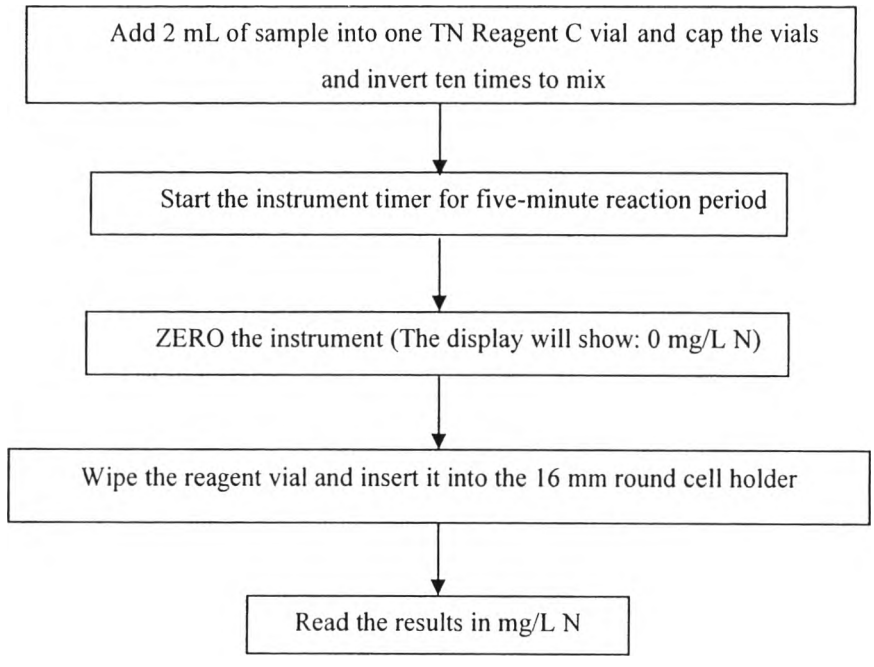

Figure D2 Procedure for analyzing amount of nitrogen (continued).

Appendix E Experiment Data of Enzymatic Hydrolysis

Table E1 Glucose produced from the hydrolysis of 40 - 60 mesh rice straw with strain A 002 at 30 °C

Time (h)	Area	Glucose (g/L)
0	3442	0.26895
1	2877	0.22480
2	3543	0.27684
3	3854	0.30114
4	4060	0.31724
5	3950	0.30864
6	4139	0.32341
7	4205	0.32857
8	4471	0.34935
9	4594	0.35896
10	4422	0.34552
11	3877	0.30294
12	3935	0.30747
15	2569	0.20073
18	2150	0.16799
24	543	0.04243

Table E2 Glucose produced from the hydrolysis of 40 - 60 mesh rice straw with strain M 015 at 30 °C

Time (h)	Area	Glucose (g/L)
0	0	0.00000
1	400451	0.14010
2	523473	0.18314
3	478558	0.16743
4	460537	0.16112
5	518710	0.18147
6	557016	0.19488
7	565976	0.19801
8	574255	0.20091
9	516448	0.18068
10	495070	0.17320
11	480148	0.16798
12	434945	0.15217
15	213990	0.07487
18	120077	0.04201
24	43298	0.01515

Table E3 Glucose produced from the hydrolysis of 60 - 80 mesh rice straw with strain A 002 at 30 °C

Time (h)	Area	Glucose (g/L)
0	3407	0.26619
1	3134	0.24488
2	4108	0.32099
3	4429	0.34607
4	4543	0.35498
5	4512	0.35256
6	4627	0.36154
7	4602	0.35959
8	4793	0.37451
9	4876	0.38100
10	3907	0.30528
11	3866	0.30208
12	3820	0.29848
15	2247	0.17557
18	1558	0.12174
24	357	0.02789

Table E4 Glucose produced from the hydrolysis of 60 – 80 mesh rice straw with strain M 015 at 30 °C

Time (h)	Area	Glucose (g/L)
0	637030	0.22287
1	576765	0.20179
2	460490	0.16111
3	528274	0.18482
4	582941	0.20395
5	608088	0.21274
6	663276	0.23205
7	643094	0.22499
8	667645	0.23358
9	712855	0.24940
10	540062	0.18894
11	531530	0.18596
12	524652	0.18355
15	293114	0.10255
18	148839	0.05207
24	88051	0.03081

Table E5 Glucose produced from the hydrolysis of < 80 mesh rice straw with strain A 002 at 30 °C

Time (h)	Area	Glucose (g/L)
0	2552	0.19944
1	1872	0.14628
2	3580	0.27975
3	3428	0.26783
4	4330	0.33836
5	4304	0.33627
6	4539	0.35468
7	4511	0.35248
8	4622	0.36115
9	4876	0.38100
10	3921	0.30640
11	2210	0.17267
12	2306	0.18019
15	2377	0.18575
18	1846	0.14423
24	478	0.03733

Table E6 Glucose produced from the hydrolysis of < 80 mesh rice straw with strain M 015 at 30 °C

Time (h)	Area	Glucose (g/L)
0	597650	0.20909
1	531361	0.18590
2	660688	0.23115
3	642636	0.22483
4	668143	0.23375
5	680290	0.23800
6	719037	0.25156
7	680805	0.23818
8	799918	0.27986
9	933885	0.32673
10	587802	0.20565
11	465452	0.16284
12	420361	0.14707
15	102268	0.03578
18	75044	0.02625
24	32189	0.01126

Table E7 Glucose produced from the hydrolysis of 40 - 60 mesh rice straw with strain A 002 at 37 °C

Time (h)	Area	Glucose (g/L)
0	2879	0.22494
1	2273	0.17761
2	1581	0.12353
3	2600	0.20316
4	3340	0.26098
5	4305	0.33638
6	4377	0.34201
7	5702	0.44554
8	6192	0.48383
9	6459	0.50469
10	6267	0.48969
11	4041	0.31575
12	2721	0.21261
15	1624	0.12689
18	461	0.03602
24	186	0.01453

Table E8 Glucose produced from the hydrolysis of 40 - 60 mesh rice straw with strain M 015 at 37 °C

Time (h)	Area	Glucose (g/L)
0	1903	0.14870
1	1768	0.13815
2	2230	0.17425
3	3007	0.23496
4	4375	0.34185
5	3737	0.29200
6	4498	0.35146
7	4512	0.35256
8	5253	0.41045
9	5035	0.39342
10	3314	0.25895
11	2294	0.17925
12	2840	0.22191
15	1702	0.13299
18	1738	0.13580
24	769	0.06009

Table E9 Glucose produced from the hydrolysis of 60 - 80 mesh rice straw with strain A 002 at 37 °C

Time (h)	Area	Glucose (g/L)
0	3601	0.28137
1	3824	0.29880
2	3528	0.27567
3	3223	0.25184
4	4067	0.31778
5	4423	0.34560
6	4610	0.36021
7	5180	0.40475
8	6569	0.51328
9	6417	0.50141
10	5806	0.45366
11	4766	0.37240
12	4897	0.38264
15	4897	0.38264
18	3132	0.24473
24	454	0.03547

Table E10 Glucose produced from the hydrolysis of 60 - 80 mesh rice straw with strain M 015 at 37 °C

Time (h)	Area	Glucose (g/L)
0	3535	0.27622
1	1662	0.12986
2	2242	0.17518
3	4784	0.37381
4	5220	0.40788
5	5349	0.41796
6	6435	0.50281
7	6675	0.52157
8	6612	0.51664
9	6945	0.54266
10	6761	0.52829
11	6722	0.52524
12	4793	0.37451
15	2427	0.18964
18	1141	0.08915
24	1012	0.07907

Table E11 Glucose produced from the hydrolysis of < 80 mesh rice straw with strain A 002 at 37 °C

Time (h)	Area	Glucose (g/L)
0	0	0.00000
1	4191	0.32747
2	4776	0.37318
3	6395	0.49969
4	6758	0.52805
5	6462	0.50492
6	6885	0.53797
7	7807	0.61002
8	7906	0.61775
9	12378	0.96718
10	6879	0.53751
11	5438	0.42491
12	3434	0.26832
15	3109	0.24293
18	2284	0.17847
24	77	0.00602

Table E12 Glucose produced from the hydrolysis of < 80 mesh rice straw with strain M 015 at 37 °C

Time (h)	Area	Glucose (g/L)
0	617	0.04821
1	2541	0.19855
2	2676	0.20910
3	3281	0.25637
4	2556	0.19972
5	2815	0.21996
6	2609	0.20386
7	4080	0.31880
8	6650	0.51961
9	6997	0.54673
10	6667	0.52094
11	6638	0.51867
12	4878	0.38115
15	2526	0.19737
18	2128	0.16628
24	471	0.03680

Table E13 Glucose and Bacteria evolution from the enzymatic hydrolysis of < 80 mesh rice straw with strain A 002 at 37 °C

Time (h)	Area	Glucose Concentration (g/L)	Nitrogen Bacteria (g/L)	Bacteria (g/L)
0	1479	0.34669	0.0340	0.30163
1	2104	0.49320	0.0420	0.3726
2	2039	0.47797	0.0480	0.42583
3	2192	0.51383	0.0520	0.46132
4	2347	0.55016	0.0540	0.47906
5	2310	0.54149	0.0600	0.53229
6	2380	0.55790	0.0680	0.60326
7	2496	0.58509	0.0720	0.63875
8	2539	0.59517	0.0820	0.72747
9	3705	0.86850	0.1000	0.88715
10	2177	0.51031	0.1060	0.94038
11	2351	0.55110	0.1100	0.97587
12	1323	0.31013	0.1140	1.01136
15	195	0.04571	0.1220	1.08233
18	109	0.02555	0.1440	1.2775
24	0	0.00000	0.1600	1.41945

Table E12 The Percentage of Cellulose Conversion from the enzymatic hydrolysis of < 80 mesh rice straw with strain A 002 at 37 °C

Time (h)	Rice Straw before hydrolysis (g)	Rice Straw after hydrolysis (g)	% Cellulose before hydrolysis	% Cellulose after hydrolysis	% Cellulose conversion
2	0.1076	0.09624	46.71	48.05	8.00
4	0.1028	0.08708	46.71	48.25	12.50
6	0.1072	0.08278	46.71	49.35	18.41
8	0.1094	0.07531	46.71	47.35	30.22
10	0.1026	0.05945	46.71	47.8	40.70
12	0.1098	0.0594	46.71	48.87	43.40
15	0.1015	0.05252	46.71	49.83	44.80
18	0.1039	0.05375	46.71	48.51	46.27
24	0.1055	0.04808	46.71	51.14	50.10

Appendix F SEM images of before and after enzymatic hydrolysis of rice straw

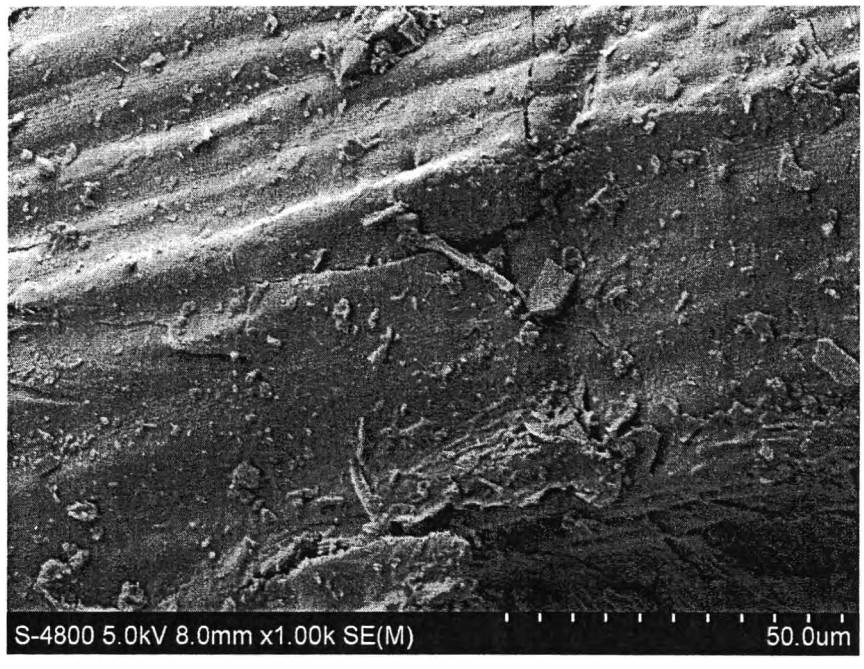

Figure F1 Scanning electron micrographs of the rice straw surface before hydrolysis.

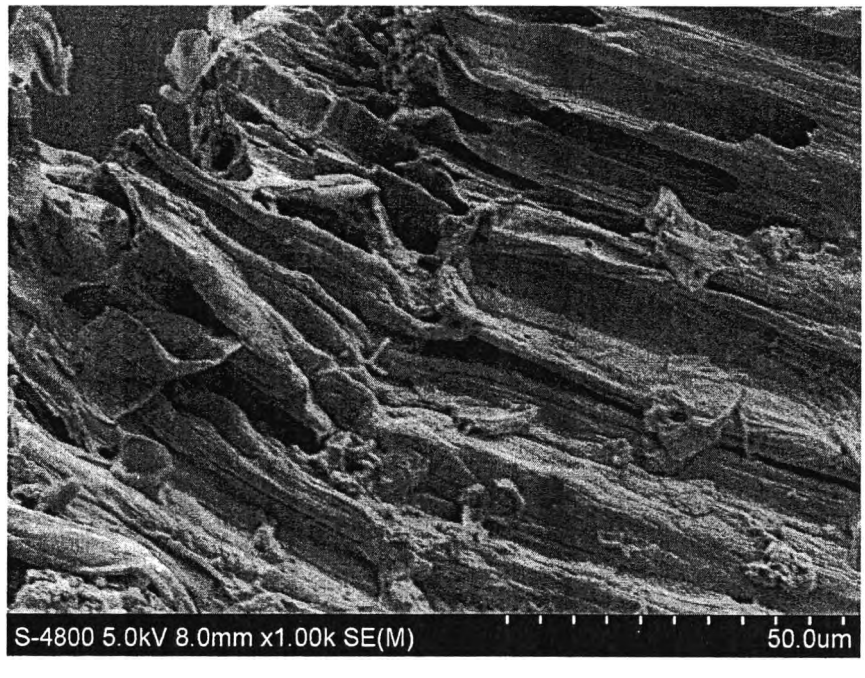

Figure F2 Scanning electron micrographs of the rice straw surface after hydrolysis of 40 – 60 mesh rice straw with strain A 002 at 30 °C.

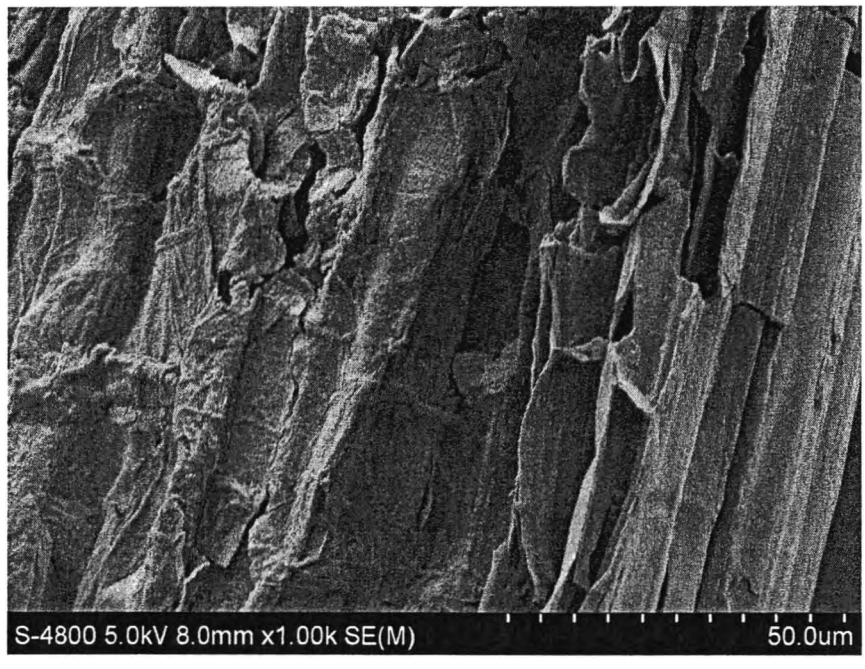

Figure F3 Scanning electron micrographs of the rice straw surface after hydrolysis of 40 - 60 mesh rice straw with strain M 015 at 30 °C.

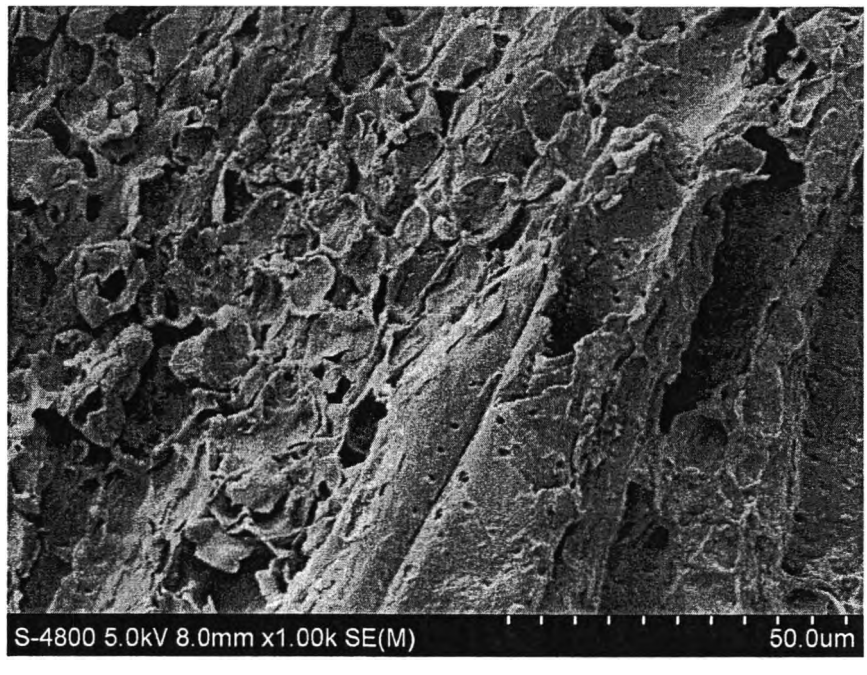

Figure F4 Scanning electron micrographs of the rice straw surface after hydrolysis of 60 – 80 mesh rice straw with strain A 002 at 30 °C.

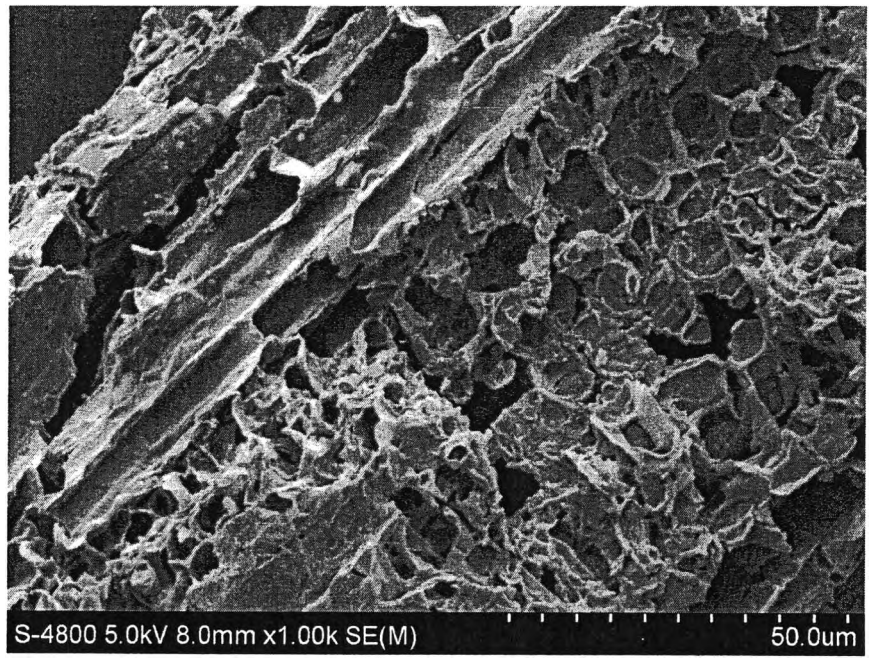

Figure F5 Scanning electron micrographs of the rice straw surface after hydrolysis of 60 - 80 mesh rice straw with strain M 015 at 30 °C.

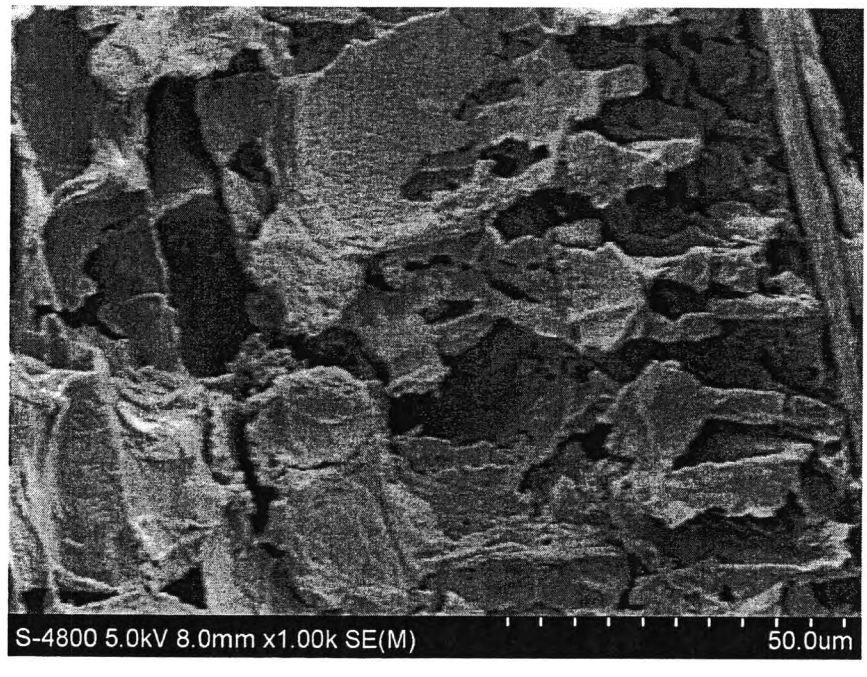

Figure F6 Scanning electron micrographs of the rice straw surface after hydrolysis of < 80 mesh rice straw with strain A 002 at 30 °C.

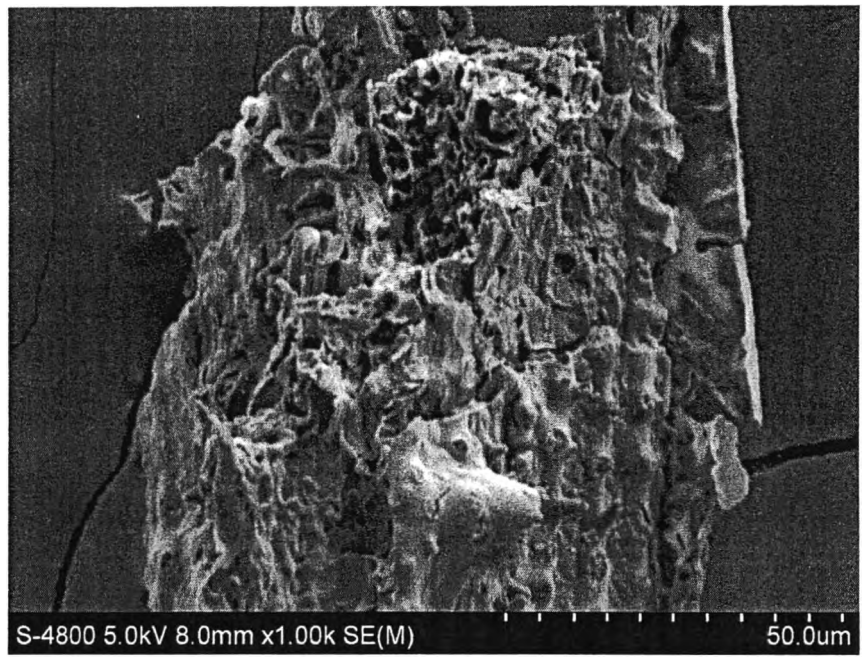

Figure F7 Scanning electron micrographs of the rice straw surface after hydrolysis of < 80 mesh rice straw with strain M 015 at 30 °C.

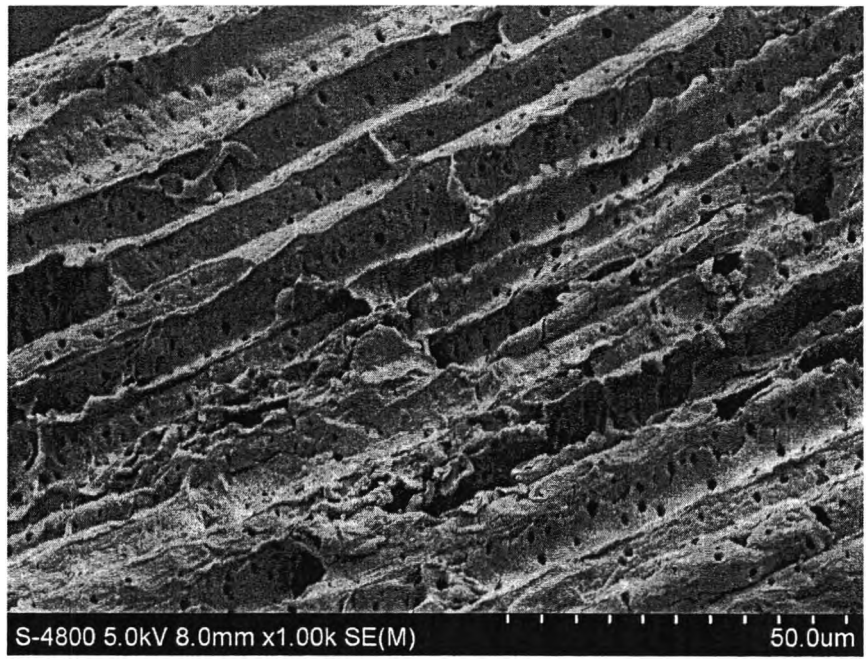

Figure F8 Scanning electron micrographs of the rice straw surface after hydrolysis of 40 - 60 mesh rice straw with strain A 002 at 37 °C.

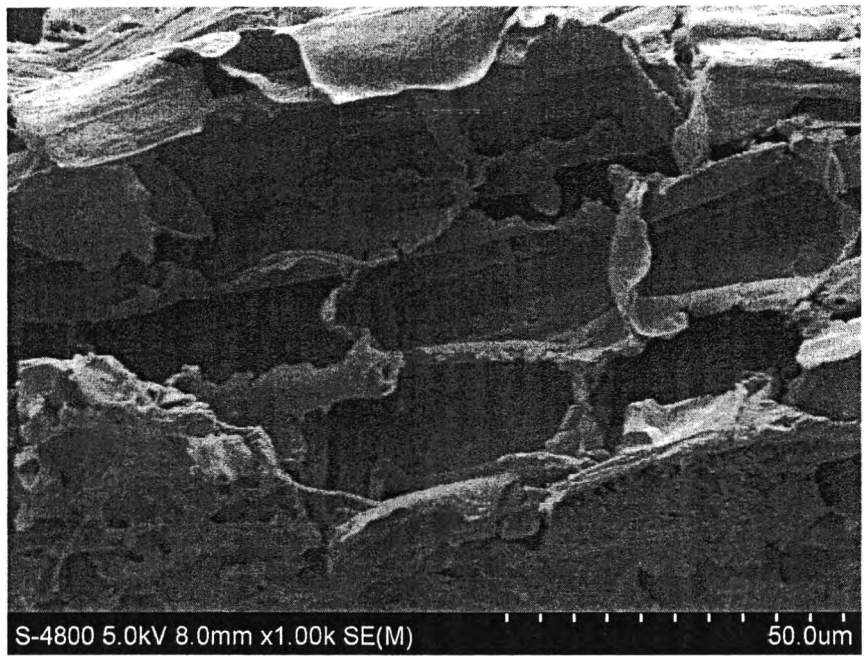

Figure F9 Scanning electron micrographs of the rice straw surface after hydrolysis of 40 – 60 mesh rice straw with strain M 015 at 37 °C.

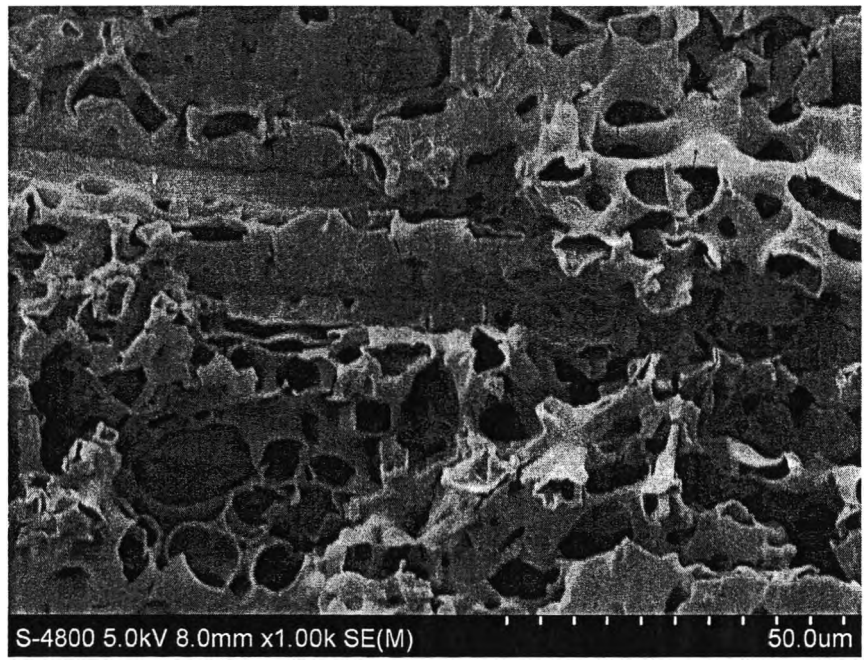

Figure F10 Scanning electron micrographs of the rice straw surface after hydrolysis of 60 – 80 mesh rice straw with strain A 002 at 37 °C.

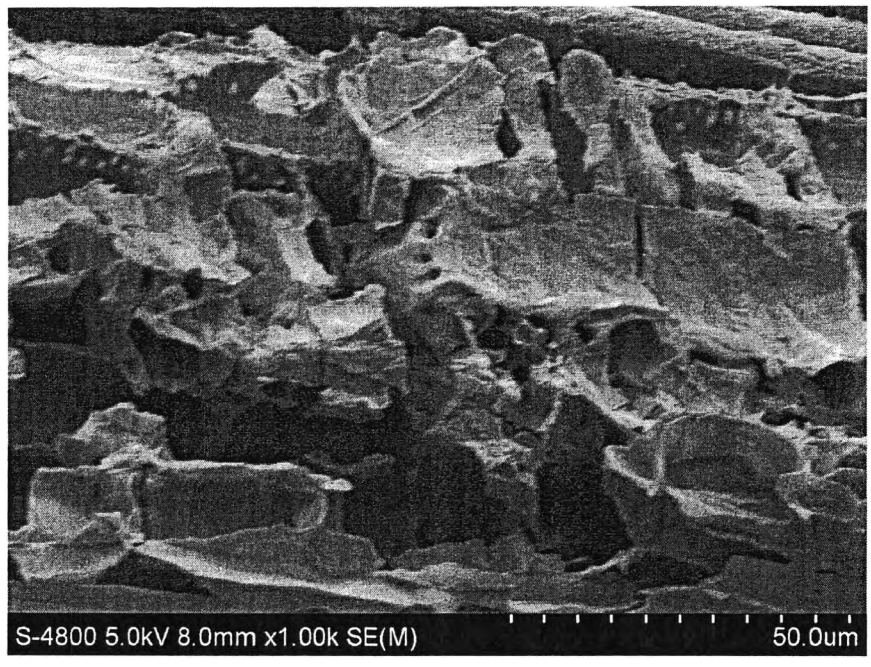

Figure F11 Scanning electron micrographs of the rice straw surface after hydrolysis of 60 - 80 mesh rice straw with strain M 015 at 37 °C.

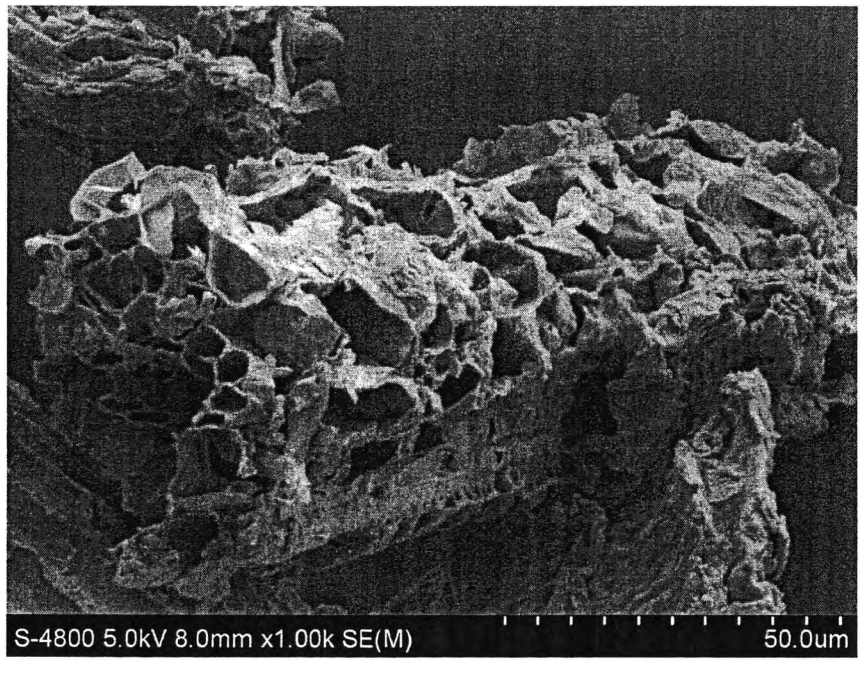

Figure F12 Scanning electron micrographs of the rice straw surface after hydrolysis of < 80 mesh rice straw with strain A 002 at 37 °C.

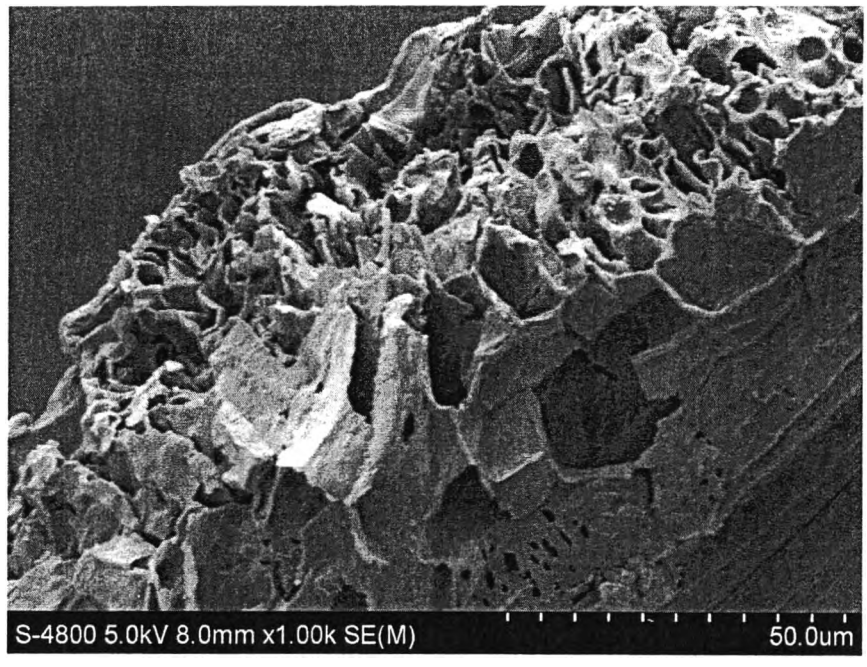

Figure F13 Scanning electron micrographs of the rice straw surface after hydrolysis of < 80 mesh rice straw with strain M 015 at 37 °C.

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1. Kerdkaew, P., Chavadej, S., and Rangsunvigit, P. (2012, April 24). Conversion of Rice Straw to Sugars by Microbial Hydrolysis. Proceedings of the 3rd Research Symposium on Petrochemical and Materials Technology and 18th PPC Symposium on Petroleum, Petrochemical, and Polymers, Bangkok, Thailand.