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APPENDICES

Appendix A Standard Calibration Curve

1. Glucose Calibration Curve

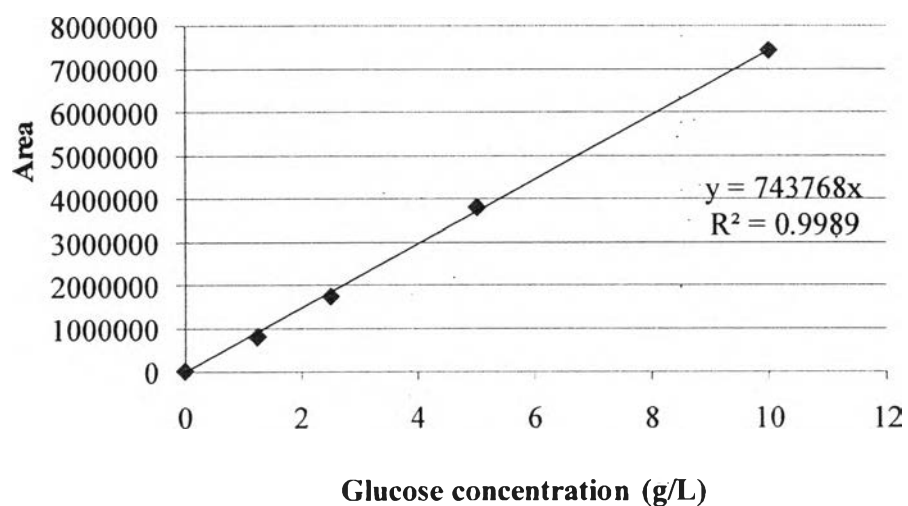


Figure A1 The relationship between glucose concentration (g/L) and area.

Table A1 Glucose calibration curve

Glucose concentration (g/L)	area(glucose)
0	0
1.25	798652
2.5	1738632
5	3808316
10	7439522

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 ₂ SO 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.

$$\text{wt. Solid} = \text{wt. Corn cob} + \text{wt. Bacteria} \quad (\text{D1})$$

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

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

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

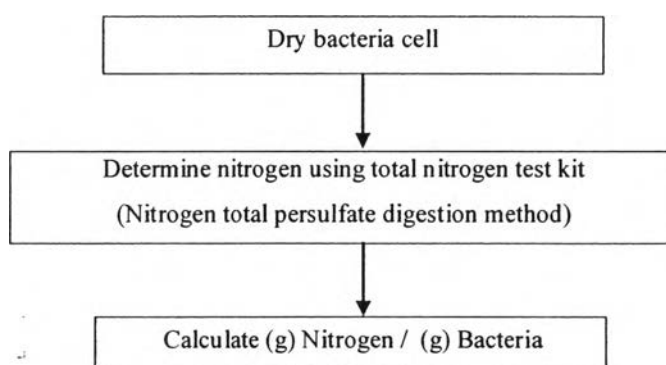


Figure D1 Diagram for determination the amount of nitrogen in bacteria.

Procedure

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

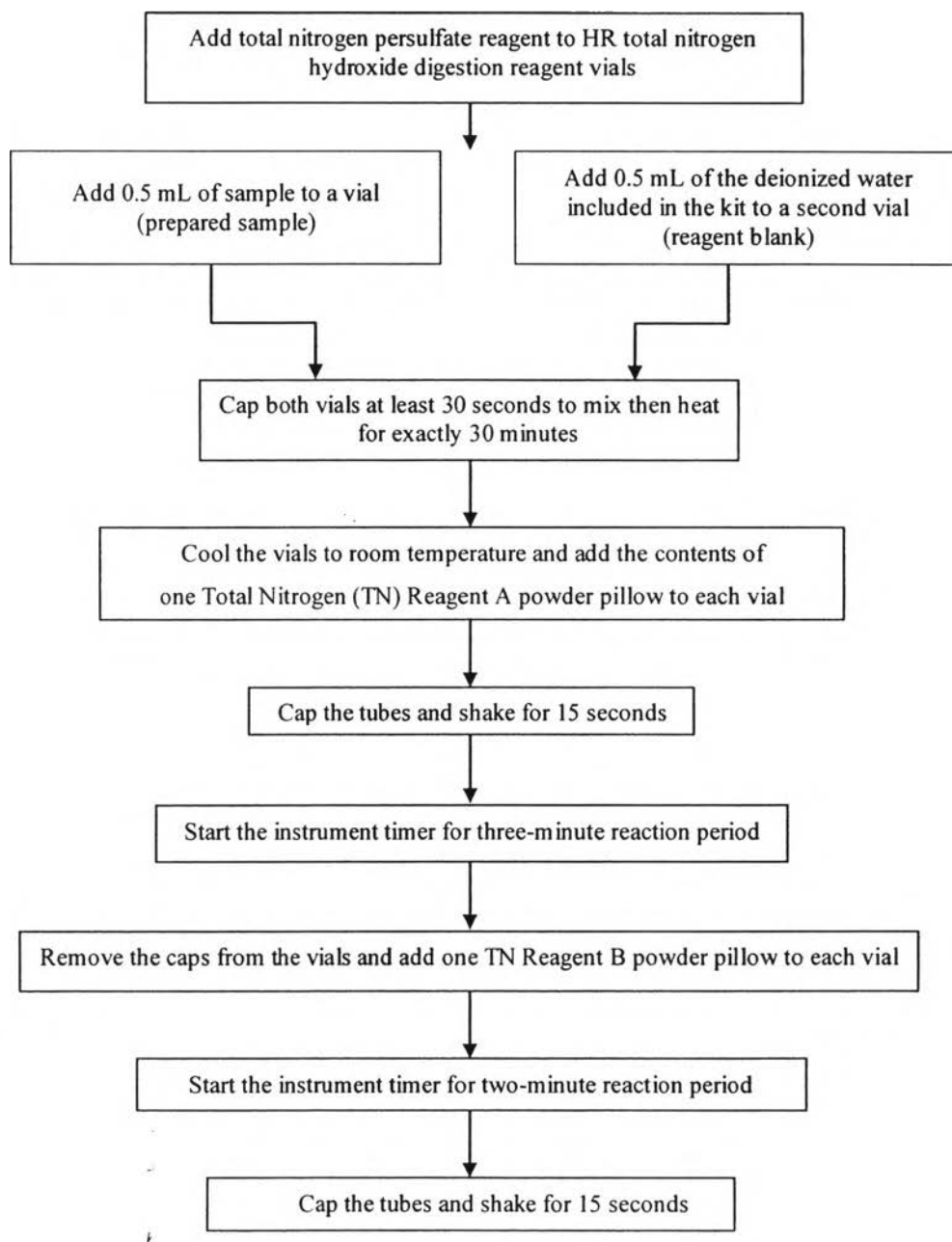


Figure D2 Procedure for analyzing amount of nitrogen.

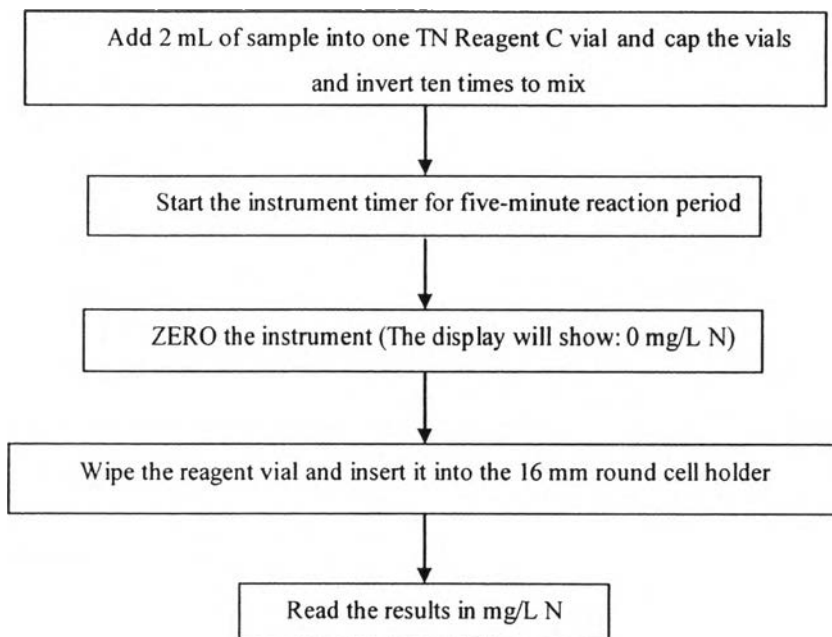


Figure D2 Procedure for analyzing amount of nitrogen (continued).

Appendix E Experiment Data of Enzymatic Hydrolysis

Table E1 Glucose produced from the hydrolysis of 60 mesh particle size corncob and strain A 002 bacteria at 37 °C.

Hr	Area	Glucose concentration (wt%)	Glucose concentration (g/L)
0	229544	0.03086	0.30862
1	116684	0.01569	0.15688
2	52858	0.00711	0.07107
3	22666	0.00305	0.03047
4	128946	0.01734	0.17337
5	247563	0.03328	0.33285
6	547499	0.07361	0.73612
7	641769	0.08629	0.86286
8	675620	0.09084	0.90837
9	804021	0.10810	1.08101
10	762416	0.10251	1.02507
11	732903	0.09854	0.98539
12	519497	0.06985	0.69847
15	160586	0.02159	0.21591
18	52666	0.00708	0.07081
24	50023	0.00673	0.06726

Table E2 Glucose produced from the hydrolysis of 40 mesh particle size corncob and strain A 002 bacteria at 37 °C.

Hr	Area	Glucose concentration (wt%)	Glucose concentration (g/L)
0	229544	0.03086	0.30862
1	36916	0.00496	0.04963
2	103369	0.01390	0.13898
3	70140	0.00943	0.09430
4	23722	0.00319	0.03189
5	233477	0.03139	0.31391
6	185312	0.02492	0.24915
7	312634	0.04203	0.42034
8	451986	0.06077	0.60770
9	725897	0.09760	0.97597
10	683000	0.09183	0.91830
11	690582	0.09285	0.92849
12	575000	0.07731	0.77309
15	554778	0.07459	0.74590
18	456580	0.06139	0.61387
24	284908	0.03831	0.38306

Table E3 Glucose produced from the hydrolysis of 60 mesh particle size comcob and strain A 002 bacteria at 30 °C.

Hr	Area	Glucose concentration (wt%)	Glucose concentration (g/L)
0	229544	0.03086	0.30862
1	249085	0.03349	0.33490
2	252909	0.03400	0.34004
3	260915	0.03508	0.35080
4	263551	0.03543	0.35435
5	318393	0.04281	0.42808
6	468493	0.06299	0.62989
7	523020	0.07032	0.70320
8	650002	0.08739	0.87393
9	703599	0.09460	0.94599
10	577300	0.07762	0.77618
11	456188	0.06133	0.61335
12	478158	0.06429	0.64289
15	401121	0.05393	0.53931
18	254973	0.03428	0.34281
24	178520	0.02400	0.24002

Table E4 Glucose produced from the hydrolysis of 40 mesh particle size corncob and strain A 002 bacteria at 30 °C.

Hr	Area	Glucose concentration (wt%)	Glucose concentration (g/L)
0	229544	0.03086	0.30862
1	172888	0.02324	0.23245
2	268048	0.03604	0.36039
3	298586	0.04015	0.40145
4	339069	0.04559	0.45588
5	353190	0.04749	0.47487
6	424803	0.05711	0.57115
7	505844	0.06801	0.68011
8	527788	0.07096	0.70961
9	662401	0.08906	0.89060
10	625263	0.08407	0.84067
11	595295	0.08004	0.80038
12	375203	0.05045	0.50446
15	375203	0.05045	0.50446
18	344951	0.04638	0.46379
24	298990	0.04020	0.40199

Table E5 Glucose produced from the hydrolysis of 60 mesh particle size corncob and strain M 015 bacteria at 37 °C.

Hr	Area	Glucose concentration (wt%)	Glucose concentration (g/L)
0	229544	0.03086	0.30862
1	306312	0.04118	0.41184
2	218858	0.02943	0.29426
3	137435	0.01848	0.18478
4	272330	0.03661	0.36615
5	267331	0.03594	0.35943
6	290858	0.03911	0.39106
7	354695	0.04769	0.47689
8	369550	0.04969	0.49686
9	391898	0.05269	0.52691
10	231627	0.03114	0.31142
11	245620	0.03302	0.33024
12	258539	0.03476	0.34761
15	267514	0.03597	0.35967
18	196442	0.02641	0.26412
24	56888	0.00765	0.07649

Table E6 Glucose produced from the hydrolysis of 40 mesh particle size corncob and strain M 015 bacteria at 37 °C.

Hr	Area	Glucose concentration (wt%)	Glucose concentration (g/L)
0	229544	0.03086	0.30862
1	255125	0.03430	0.34302
2	176015	0.02367	0.23665
3	172123	0.02314	0.23142
4	211878	0.02849	0.28487
5	322648	0.04338	0.43380
6	328453	0.04416	0.44161
7	328017	0.04410	0.44102
8	359922	0.04839	0.48392
9	365885	0.04919	0.49193
10	331961	0.04463	0.44632
11	325514	0.04377	0.43766
12	300236	0.04037	0.40367
15	226688	0.03048	0.30478
18	163065	0.02192	0.21924
24	135891	0.01827	0.18271

Table E7 Glucose produced from the hydrolysis of 60 mesh particle size corncob and strain M 015 bacteria at 30 °C.

Hr	Area	Glucose concentration (wt%)	Glucose concentration (g/L)
0	229544	0.03086	0.30862
1	58330	0.00784	0.07842
2	59856	0.00805	0.08048
3	60012	0.00807	0.08069
4	61792	0.00831	0.08308
5	88924	0.01196	0.11956
6	95865	0.01289	0.12889
7	70140	0.00943	0.09430
8	84075	0.01130	0.11304
9	225050	0.03026	0.30258
10	185312	0.02492	0.24915
11	103369	0.01390	0.13898
12	67168	0.00903	0.09031
13	23336	0.00314	0.03138
14	50680	0.00681	0.06814
15	25756	0.00346	0.03463
16	29520	0.00397	0.03969
18	18965	0.00255	0.02550
24	13334	0.00179	0.01793

Table E8 Glucose produced from the hydrolysis of 40 mesh particle size corncob and strain M 015 bacteria at 30 °C.

Hr	Area	Glucose concentration (wt%)	Glucose concentration (g/L)
0	229544	0.03086	0.30862
1	11000	0.00148	0.01479
2	13560	0.00182	0.01823
3	14520	0.00195	0.01952
4	15349	0.00206	0.02064
5	15902	0.00214	0.02138
6	17390	0.00234	0.02338
7	70207	0.00944	0.09439
8	64243	0.00864	0.08638
9	180628	0.02429	0.24286
10	145200	0.01952	0.19522
11	108328	0.01456	0.14565
12	104772	0.01409	0.14087
13	120690	0.01623	0.16227
14	120036	0.01614	0.16139
15	99958	0.01344	0.13439
16	78596	0.01057	0.10567
18	56000	0.00753	0.07529
24	52223	0.00702	0.07021

Table E9 Bacteria evolution from the enzymatic hydrolysis of 60 mesh size corncob with strain A 002 bacteria at 37 °C.

Hr	Total bacterial (g/L)	Solid(g)	Nitrogen bacteria (g/L)	Bacterial (g/L)
0	10.3	10.244	0.056	0.496774
2	14.2	14.124	0.076	0.674194
3	23.1	23	0.1	0.887097
5	14.4	14.278	0.122	1.082258
7	14	13.84	0.16	1.419355
8	9.9	9.708	0.192	1.703226
9	10	9.8	0.2	1.774194
12	16.6	16.34	0.26	2.306452
18	24.4	24.1	0.3	2.66129
24	14.9	14.582	0.318	2.820968

Table E10 Bacteria evolution from the enzymatic hydrolysis of 40 mesh size corncob with strain A 002 bacteria at 37 °C.

Hr	Total bacterial (g/L)	Solid(g)	Nitrogen bacteria (g/L)	Bacterial (g/L)
0	6.2	6.142	0.058	0.514516
3	10.3	10.23	0.07	0.620968
5	11.5	11.41	0.09	0.798387
6	11.7	11.592	0.108	0.958065
9	7.2	7.004	0.196	1.73871
12	8	7.798	0.202	1.791935
18	7.7	7.482	0.218	1.933871
24	15.6	15.404	0.196	1.73871

Table E11 Bacteria evolution from the enzymatic hydrolysis of 60 mesh size corncob with strain A 002 bacteria at 30 °C.

Hr	Total bacterial (g/L)	Solid(g)	Nitrogen bacteria (g/L)	Bacterial (g/L)
0	4.9	4.825	0.075	0.665323
2	10.3	10.14	0.16	1.419355
5	6	5.824	0.176	1.56129
7	8.8	8.6	0.2	1.774194
9	7.6	7.36	0.24	2.129032
12	9	8.75	0.25	2.217742
18	9.5	9.252	0.248	2.2
24	9.4	9.16	0.24	2.129032

Table E12 Bacteria evolution from the enzymatic hydrolysis of 40 mesh size corncob with strain A 002 bacteria at 30 °C.

Hr	Total bacterial (g/L)	Solid(g)	Nitrogen bacteria (g/L)	Bacterial (g/L)
0	8.1	7.998	0.102	0.904839
3	10.4	10.242	0.158	1.401613
7	2.6	2.466	0.134	1.18871
9	5.7	5.52	0.18	1.596774
12	4	3.832	0.168	1.490323
14	5.7	5.492	0.208	1.845161
16	7.4	7.186	0.214	1.898387
18	4	3.782	0.218	1.933871
24	5.9	5.7	0.2	1.774194

Table E13 Bacteria evolution from the enzymatic hydrolysis of 60 mesh size corn cob with strain M 015 bacteria at 37 °C.

Hr	Total bacterial (g/L)	Solid(g)	Nitrogen bacteria (g/L)	Bacterial (g/L)
0	1.2	1.131	0.069	0.432997
3	4.4	4.290	0.110	0.694737
7	1	0.820	0.180	1.136842
10	2.7	2.540	0.160	1.010526
12	2.9	2.706	0.194	1.225263
16	3.1	2.856	0.244	1.541053
18	2.9	2.656	0.244	1.541053
24	8.3	8.054	0.246	1.553684

Table E14 Bacteria evolution from the enzymatic hydrolysis of 40 mesh size corn cob with strain M 015 bacteria at 37 °C.

Hr	Total bacterial (g/L)	Solid(g)	Nitrogen bacteria (g/L)	Bacterial (g/L)
0	2	1.963	0.1	0.631579
3	1.1	0.962	0.138	0.871579
4	4.6	4.45	0.15	0.947368
6	12	11.742	0.258	1.629474
7	11.6	11.296	0.304	1.92
9	30	29.688	0.312	1.970526
12	23.2	22.88	0.32	2.021053
18	11	10.694	0.306	1.932632
24	7.6	7.286	0.314	1.983158

Table E15 Bacteria evolution from the enzymatic hydrolysis of 60 mesh size corn cob with strain M 015 bacteria at 30 °C.

Hr	Total bacterial (g/L)	Solid(g)	Nitrogen bacteria(g/L)	Bacterial (g/L)
0	13	12.948	0.052	0.328421
1	16.3	16.14	0.16	1.010526
3	53.7	53.46	0.24	1.515789
4	27.3	27.054	0.246	1.553684
9	13.7	13.4	0.3	1.894737
12	51.5	51.25	0.25	1.578947
18	28.2	27.92	0.28	1.768421
24	22.7	22.428	0.272	1.717895

Table E16 Bacteria evolution from the enzymatic hydrolysis of 40 mesh size corn cob with strain M 015 bacteria at 30 °C.

Hr	Total bacterial (g/L)	Solid(g)	Nitrogen bacteria(g/L)	Bacterial (g/L)
0	3.6	3.582	0.018	0.113684
1	34.9	34.784	0.116	0.732632
3	7.5	7.196	0.304	1.92
4	2.7	2.388	0.312	1.970526
9	27	26.58	0.42	2.652632
10	37.8	37.358	0.442	2.791579
18	45.2	44.748	0.452	2.854737
24	30.3	29.856	0.444	2.804211

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