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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.

2. Cellobiose Calibration Curve



Figure A2 The relationship between cellobiose concentration (g/L) and area.

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

 Table A1
 Glucose calibration curve

 Table A2
 Cellobiose calibration curve

Cellobiose concentration (g/L)	area(cellobiose)
0	0
0.125	1656.000
0.25	3796.000
0.5	8631.456
1.0	19405.130
1.5	28096.500

## 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 <sub>2</sub> SO <sub>4</sub> 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.

wt. Solid = wt. Corncob + wt. Bacteria (D1)

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

wt. Bacteria = <u>g Nitrogen contained in sample</u> (D2) (g Nitrogen / 1 g Bacteria)

#### 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 and cellobiose remaining from the hydrolysis of 40 mesh particle size cassava residue and strain A 002bacteria at 30 °C.

Timo	Glucose			Cellobiose		
(h)	Area	Concentration (wt%)	Concentration (g/L)	Area	Concentration (wt%)	Concentration (g/L)
0	3316.3690	0.0259	0.2591	15901.3250	0.0849	0.8489
1	4222.7410	0.0330	0.3300	18457.6190	0.0985	0.9854
2	5030.4275	0.0393	0.3931	18094.0710	0.0966	0.9660
3	5702.5230	0.0446	0.4456	19351.8255	0.1033	1.0331
4	5992.4415	0.0468	0.4682	18271.8905	0.0975	0.9755
5	1794.3260	0.0140	0.1402	17561.0025	0.0938	0.9375
6	2453.2375	0.0192	0.1917	17670.0130	0.0943	0.9434
7	2297.5125	0.0180	0.1795	16716.9730	0.0892	0.8925
8	2005.6840	0.0157	0.1567	14794.6665	0.0790	0.7898
9	4294.0990	0.0336	0.3355	16212.2530	0.0866	0.8655
10	6949.0000	0.0543	0.5430	15636.2015	0.0835	0.8348
11	5107.0300	0.0399	0.3990	15294.8990	0.0817	0.8166
12	5053.8310	0.0395	0.3949	14456.2310	0.0772	0.7718
15	4875.0000	0.0381	0.3809	9042.5585	0.0483	0.4828
18	4900.0000	0.0383	0.3829	7265.2570	0.0388	0.3879
24	3122.0721	0.0244	0.2440	4973.5560	0.0266	0.2655

**Table E2** Glucose produced and cellobiose remaining from the hydrolysis of 40 mesh particle size cassava residue and strain M 015bacteria at 30 °C.

	Glucose			Cellobiose		
(h)	Area	Concentration (wt%)	Concentration (g/L)	Area	Concentration (wt%)	Concentration (g/L)
0	327.8985	0.0026	0.0256	17896.4750	0.0955	0.9554
1	3074.2440	0.0240	0.2402	20693.0230	0.1105	1.1047
2	3495.7680	0.0273	0.2731	20698.9590	0.1105	1.1051
3	4306.2895	0.0336	0.3365	20026.1170	0.1069	1.0691
4	4980.7690	0.0389	0.3892	19136.0650	0.1022	1.0216
5	5088.2790	0.0398	0.3976	18402.7980	0.0982	0.9825
6	4708.4040	0.0368	0.3679	16887.7890	0.0902	0.9016
7	4798.6101	0.0375	0.3750	16190.8200	0.0864	0.8644
8	4167.0288	0.0326	0.3256	15272.0015	0.0815	0.8153
9	4313.1390	0.0337	0.3370	14074.9875	0.0751	0.7514
10	5293.7380	0.0414	0.4136	12631.6430	0.0674	0.6744
11	4319.0920	0.0337	0.3375	11855.0755	0.0633	0.6329
12	4287.7895	0.0335	0.3350	10809.9970	0.0577	0.5771
15	4312.3770	0.0337	0.3370	8589.7250	0.0459	0.4586
18	3213.9560	0.0251	0.2511	5948.6280	0.0318	0.3176
24	817.8740	0.0064	0.0639	2129.4925	0.0114	0.1137

**Table E3** Glucose produced and cellobiose remaining from the hydrolysis of 60 mesh particle size cassava residue and strain A 002bacteria at 30 °C.

<b>T</b> .	Glucose			lucose Cellobiose		
(h)	Area	Concentration (wt%)	Concentration (g/L)	Area	Concentration (wt%)	Concentration (g/L)
0	999.0060	0.0078	0.0781	13895.6110	0.0742	0.7419
1	5808.6720	0.0454	0.4539	15647.0000	0.0835	0.8354
2	2331.6440	0.0182	0.1822	18846.9420	0.1006	1.0062
3	2763.7281	0.0216	0.2160	21086.4550	0.1126	1.1258
4	4837.6680	0.0378	0.3780	18944.3625	0.1011	1.0114
5	5275.1155	0.0412	0.4122	18895.8860	0.1009	1.0088
6	4488.1306	0.0351	0.3507	17843.1750	0.0953	0.9526
7	5376.4720	0.0420	0.4201	18263.7740	0.0975	0.9751
8	5814.5420	0.0454	0.4543	17378.6010	0.0928	0.9278
9	5918.1850	0.0462	0.4624	16489.7820	0.0880	0.8803
10	10466.2860	0.0818	0.8178	15023.2900	0.0802	0.8021
11	8808.6074	0.0688	0.6883	12193.8555	0.0651	0.6510
12	6614.9023	0.0517	0.5169	8559.5560	0.0457	0.4570
15	4388.4342	0.0343	0.3429	7122.7270	0.0380	0.3803
18	2007.4225	0.0157	0.1569	3408.8080	0.0182	0.1820
24	1611.1200	0.0126	0.1259	1438.6840	0.0077	0.0768

**Table E4** Glucose produced and cellobiose remaining from the hydrolysis of 60 mesh particle size cassava residue and strain M 015bacteria at 30 °C.

	Glucose			Cellobiose		
(h)	Area	Concentration (wt%)	Concentration (g/L)	Area	Concentration (wt%)	Concentration (g/L)
0	3541.5050	0.0277	0.2767	19475.6655	0.1040	1.0398
1	4179.8680	0.0327	0.3266	20694.5750	0.1105	1.1048
2	4671.5025	0.0365	0.3650	22066.0540	0.1178	1.1780
3	4963.5285	0.0388	0.3878	19327.2925	0.1032	1.0318
4	5318.4910	0.0416	0.4156	17751.9585	0.0948	0.9477
5	5468.4270	0.0427	0.4273	16958.6330	0.0905	0.9054
6	5452.6570	0.0426	0.4261	16724.7075	0.0893	0.8929
7	5414.0659	0.0423	0.4230	16222.0075	0.0866	0.8661
8	5411.8470	0.0423	0.4229	15098.4540	0.0806	0.8061
9	5963.0520	0.0466	0.4659	13436.0270	0.0717	0.7173
10	5638.9995	0.0441	0.4406	12742.4435	0.0680	0.6803
11	5760.6190	0.0450	0.4501	12112.6160	0.0647	0.6467
12	5561.8828	0.0435	0.4346	11082.1230	0.0592	0.5916
15	5168.6150	0.0404	0.4039	8835.3490	0.0472	0.4717
18	4140.7929	0.0324	0.3236	4756.9270	0.0254	0.2540
24	2270.0330	0.0177	0.1774	2270.7810	0.0121	0.1212

**Table E5** Glucose produced and cellobiose remaining from the hydrolysis of 80 mesh particle size cassava residue and strain A 002 bacteria at 30 °C.

Time	Glucose			Cellobiose		
(h)	Area	Concentration (wt%)	Concentration (g/L)	Area	Concentration (wt%)	Concentration (g/L)
0	1034.0000	0.0081	0.0808	19190.2675	0.1025	1.0245
1	1674.0000	0.0131	0.1308	19461.5050	0.1039	1.0390
2	4697.0000	0.0367	0.3670	20562.4440	0.1098	1.0978
3	5986.0000	0.0468	0.4677	20508.5510	0.1095	1.0949
4	7864.0000	0.0614	0.6145	8567.0000	0.0457	0.4574
5	10385.0000	0.0811	0.8115	17545.2575	0.0937	0.9367
6	9885.0000	0.0772	0.7724	16903.7650	0.0902	0.9024
7	18485.0000	0.1444	1.4444	3186.3590	0.0170	0.1701
8	9587.0000	0.0749	0.7491	15928.9990	0.0850	0.8504
9	7485.0000	0.0585	0.5849	15551.9005	0.0830	0.8303
10	7100.0000	0.0555	0.5548	14761.0000	0.0788	0.7881
11	6847.0000	0.0535	0.5350	14579.0810	0.0778	0.7783
12	5783.2695	0.0452	0.4519	13660.3520	0.0729	0.7293
15	5209.0000	0.0407	0.4070	10979.5900	0.0586	0.5862
18	4586.0000	0.0358	0.3583	6541.5985	0.0349	0.3492
24	4493.0000	0.0351	0.3511	3926.5350	0.0210	0.2096

		Glucose		Cellobiose		
(h)	Area	Concentration (wt%)	Concentration (g/L)	Area	Concentration (wt%)	Concentration (g/L)
0	5823.8579	0.0455	0.4551	3226.7850	0.0172	0.1723
1	5227.9830	0.0409	0.4085	18200.7290	0.0972	0.9717
2	5382.0000	0.0421	0.4205	19307.7730	0.1031	1.0308
3	7022.0000	0.0549	0.5487	20323.1255	0.1085	1.0850
4	7747.0133	0.0605	0.6053	16302.6965	0.0870	0.8704
5	7553.0000	0.0590	0.5902	17401.4020	0.0929	0.9290
6	7695.0000	0.0601	0.6013	15780.8060	0.0842	0.8425
7	7650.0000	0.0598	0.5977	15451.6810	0.0825	0.8249
8	12481.0000	0.0975	0.9752	13656.9490	0.0729	0.7291
9	7411.0000	0.0579	0.5791	12031.6555	0.0642	0.6423
10	7291.0000	0.0570	0.5697	10873.1155	0.0580	0.5805
11	6508.5509	0.0509	0.5086	8695.6100	0.0464	0.4642
12	5882.9846	0.0460	0.4597	2696.6310	0.0144	0.1440
15	6293.0000	0.0492	0.4917	6369.4985	0.0340	0.3401
18	5442.0000	0.0425	0.4252	3081.8680	0.0165	0.1645
24	2506.6163	0.0196	0.1959	142.4115	0.0008	0.0076

**Table E6** Glucose produced and cellobiose remaining from the hydrolysis of 80 mesh particle size cassava residue and strain M 015bacteria at 30 °C.

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**Table E7** Glucose produced and cellobiose remaining from the hydrolysis of 40 mesh particle size cassava residue and strain A 002bacteria at 37 °C.

	Glucose			Cellobiose		
(h)	Area	Concentration (wt%)	Concentration (g/L)	Area	Concentration (wt%)	Concentration (g/L)
0	280.6601	0.0022	0.0219	2726.9240	0.0146	0.1456
1	325.2065	0.0025	0.0254	3854.6285	0.0206	0.2058
2	754.8240	0.0059	0.0590	4467.7755	0.0239	0.2385
3	1039.0420	0.0081	0.0812	4667.9170	0.0249	0.2492
4	1033.1500	0.0081	0.0807	3181.6765	0.0170	0.1699
5	198.7970	0.0016	0.0155	3856.2540	0.0206	0.2059
6	76.1810	0.0006	0.0060	512.3420	0.0027	0.0274
7	443.1860	0.0035	0.0346	1770.3585	0.0095	0.0945
8	1004.7530	0.0079	0.0785	2912.8880	0.0156	0.1555
9	1281.2275	0.0100	0.1001	2853.3590	0.0152	0.1523
10	963.4770	0.0075	0.0753	2469.5215	0.0132	0.1318
11	888.3470	0.0069	0.0694	2205.7220	0.0118	0.1178
12	904.8840	0.0071	0.0707	2023.7515	0.0108	0.1080
15	576.0625	0.0045	0.0450	1170.3540	0.0062	0.0625
18	330.2280	0.0026	0.0258	536.5410	0.0029	0.0286
24	74.3880	0.0006	0.0058	83.0030	0.0004	0.0044

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**Table E8** Glucose produced and cellobiose remaining from the hydrolysis of 40 mesh particle size cassava residue and strain M 015bacteria at 37 °C.

<b></b>	Glucose			Cellobiose		
(h)	Area	Concentration (wt%)	Concentration (g/L)	Area	Concentration (wt%)	Concentration (g/L)
0	705.6430	0.0055	0.0551	4288.9950	0.0229	0.2290
1	561.1860	0.0044	0.0438	4470.1940	0.0239	0.2387
2	927.8740	0.0073	0.0725	3420.4290	0.0183	0.1826
3	852.0165	0.0067	0.0666	1694.3780	0.0090	0.0905
4	790.7900	0.0062	0.0618	4008.0755	0.0214	0.2140
5	838.6170	0.0066	0.0655	3805.1000	0.0203	0.2031
6	522.0800	0.0041	0.0408	2347.6490	0.0125	0.1253
7	558.8550	0.0044	0.0437	1111.3445	0.0059	0.0593
8	807.2650	0.0063	0.0631	2423.6700	0.0129	0.1294
9	1118.4715	0.0087	0.0874	2582.6060	0.0138	0.1379
10	690.8635	0.0054	0.0540	1136.1130	0.0061	0.0607
11	685.6750	0.0054	0.0536	953.5210	0.0051	0.0509
12	701.1055	0.0055	0.0548	1647.1970	0.0088	0.0879
15	797.9695	0.0062	0.0624	1310.5905	0.0070	0.0700
18	671.6045	0.0052	0.0525	661.2795	0.0035	0.0353
24	149.4980	0.0012	0.0117	121.4260	0.0006	0.0065

**Table E9** Glucose produced and cellobiose remaining from the hydrolysis of 60 mesh particle size cassava residue and strain A 002bacteria at 37 °C.

	Glucose			Cellobiose		
(h)	Area	Concentration (wt%)	Concentration (g/L)	Area	Concentration (wt%)	Concentration (g/L)
0	728.9741	0.0057	0.0570	3542.5400	0.0189	0.1891
1	830.2460	0.0065	0.0649	5498.1600	0.0294	0.2935
2	657.3980	0.0051	0.0514	4522.4235	0.0241	0.2414
3	307.5170	0.0024	0.0240	5325.4340	0.0284	0.2843
4	348.2340	0.0027	0.0272	5679.9420	0.0303	0.3032
5	339.4020	0.0027	0.0265	6226.1990	0.0332	0.3324
6	323.3040	0.0025	0.0253	2780.5390	0.0148	0.1484
7	329.2180	0.0026	0.0257	4306.6620	0.0230	0.2299
8	77.4630	0.0006	0.0061	4401.8465	0.0235	0.2350
9	58.7680	0.0005	0.0046	4163.3820	0.0222	0.2223
10	1935.1640	0.0151	0.1512	3518.6210	0.0188	0.1879
11	1769.4856	0.0138	0.1383	2853.4850	0.0152	0.1523
12	1416.0755	0.0111	0.1106	1984.2510	0.0106	0.1059
15	1162.5565	0.0091	0.0908	1215.0340	0.0065	0.0649
18	793.2350	0.0062	0.0620	418.5935	0.0022	0.0223
24	161.2400	0.0013	0.0126	88.6785	0.0005	0.0047

**Table E10** Glucose produced and cellobiose remaining from the hydrolysis of 60 mesh particle size cassava residue and strain M 015 bacteria at 37 °C.

Time	Glucose			Cellobiose		
(h)	Area	Concentration (wt%)	Concentration (g/L)	Area	Concentration (wt%)	Concentration (g/L)
0	423.4070	0.0033	0.0331	3281.3390	0.0175	0.1752
1	250.9850	0.0020	0.0196	2242.9850	0.0120	0.1197
2	612.8450	0.0048	0.0479	612.8450	0.0033	0.0327
3	872.5370	0.0068	0.0682	4418.8730	0.0236	0.2359
4	696.4720	0.0054	0.0544	3139.5635	0.0168	0.1676
5	1001.4440	0.0078	0.0783	179.5295	0.0010	0.0096
6	907.4780	0.0071	0.0709	3552.7370	0.0190	0.1897
7	943.2490	0.0074	0.0737	3298.3115	0.0176	0.1761
8	815.3555	0.0064	0.0637	3077.8200	0.0164	0.1643
9	895.4350	0.0070	0.0700	2536.4750	0.0135	0.1354
10	1401.5900	0.0110	0.1095	2745.0660	0.0147	0.1466
11	957.6445	0.0075	0.0748	2517.5130	0.0134	0.1344
12	938.7760	0.0073	0.0734	2231.7390	0.0119	0.1191
15	681.0075	0.0053	0.0532	1375.6800	0.0073	0.0734
18	539.1530	0.0042	0.0421	1196.1100	0.0064	0.0639
24	369.3310	0.0029	0.0289	400.7210	0.0021	0.0214

**Table E12** Glucose produced and cellobiose remaining from the hydrolysis of 80 mesh particle size cassava residue and strain M 015bacteria at 37 °C.

	Glucose			Cellobiose		
(h)	Area	Concentration (wt%)	Concentration (g/L)	Area	Concentration (wt%)	Concentration (g/L)
0	365.7810	0.0029	0.0286	3662.9725	0.0196	0.1956
1	381.2860	0.0030	0.0298	5193.4840	0.0277	0.2773
2	678.5400	0.0053	0.0530	1748.8400	0.0093	0.0934
3	593.6500	0.0046	0.0464	1813.2395	0.0097	0.0968
4	1028.0390	0.0080	0.0803	4034.3620	0.0215	0.2154
5	462.2100	0.0036	0.0361	2228.7970	0.0119	0.1190
6	732.6700	0.0057	0.0572	3082.2340	0.0165	0.1646
7	2287.0815	0.0179	0.1787	1983.8545	0.0106	0.1059
8	2585.0425	0.0202	0.2020	2534.6535	0.0135	0.1353
9	1495.6000	0.0117	0.1169	678.3110	0.0036	0.0362
10	1186.4560	0.0093	0.0927	1049.7565	0.0056	0.0560
11	964.6790	0.0075	0.0754	3860.3690	0.0206	0.2061
12	653.7800	0.0051	0.0511	3659.4460	0.0195	0.1954
15	798.3560	0.0062	0.0624	347.8120	0.0019	0.0186
18	467.8300	0.0037	0.0366	908.4530	0.0048	0.0485
24	216.0670	0.0017	0.0169	231.2610	0.0012	0.0123

Time (h)	Glucose Concentration (g/L)	Nitrogen bacteria (g/L)	Bacterial (g/L)
0	0.0808	0.1120	0.9936
2	0.3670	0.1580	1.4017
4	0.6145	0.1700	1.5082
6	0.7724	0.1800	1.5969
8	0.7491	0.1900	1.6856
10	0.5548	0.1720	1.5259
12	0.4519	0.1900	1.6856
15	0.4070	0.2020	1.7921
18	0.3583	0.2380	2.1114
24	0.3511	0.2400	2.1292

**Table E13** Glucose and Bacteria evolution from the enzymatic hydrolysis of 80mesh size cassava residue with strain A 002 bacteria at 30 °C.

Appendix F SEM images of before and after enzymatic hydrolysis of cassava residue



Figure F1 Scanning electron micrographs of the cassava residue surface before hydrolysis.



Figure F2 Scanning electron micrographs of the cassava residue surface after hydrolysis 40 mesh cassava residue with strain A 002 at 30  $^{\circ}$ C.



Figure F3 Scanning electron micrographs of the cassava residue surface after hydrolysis 40 mesh cassava residue with strain M 015 at 30  $^{\circ}$ C.



Figure F4 Scanning electron micrographs of the cassava residue surface after hydrolysis 60 mesh cassava residue with strain A 002 at 30  $^{\circ}$ C.



Figure F5 Scanning electron micrographs of the cassava residue surface after hydrolysis 60 mesh cassava residue with strain M 015 at 30  $^{\circ}$ C.



Figure F6 Scanning electron micrographs of the cassava residue surface after hydrolysis 80 mesh cassava residue with strain A 002 at 30  $^{\circ}$ C.



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

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Figure F8 Scanning electron micrographs of the cassava residue surface after hydrolysis 40 mesh cassava residue with strain A 002 at 37  $^{\circ}$ C.



Figure F9 Scanning electron micrographs of the cassava residue surface after hydrolysis 40 mesh cassava residue with strain M 015 at 37 °C.



Figure F10 Scanning electron micrographs of the cassava residue surface after hydrolysis 60 mesh cassava residue with strain A 002 at 37  $^{\circ}$ C.



Figure F11 Scanning electron micrographs of the cassava residue surface after hydrolysis 60 mesh cassava residue with strain M 015 at 37  $^{\circ}$ C.



Figure F12 Scanning electron micrographs of the cassava residue surface after hydrolysis 80 mesh cassava residue with strain A 002 at 37  $^{\circ}$ C.



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

#### **CURRICULUM VITAE**

Name: Ms. Pitcha Wongskeo

Date of Birth: March 15, 1988

Nationality: Thai

## **University Education:**

2005-2008 Bachelor Degree of Chemical Engineering, Department of Chemical Engineering, Faculty of Engineering, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

## Work Experience:

2009 Position: Process Engineer (Student Internship) Company: Esso (Thailand) Public Co., Ltd

### **Proceedings:**

- 1. Wongskeo P., Rangsunvigit P., and Chavadej, S., (2012, April 11-13) Glucose Evolution from Cassava Residue by Microbial Hydrolysis Using Bacteria Isolated from Thai Higher Termites. Poster presentation at ICCBEE 2012 : International Conference on Chemical, Biological and Environmental Engineering, Venice, Italy.
- Wongskeo, P., Rangsunvigit, P., and Chavadej, S., (2012, April 24) Glucose Evolution from Cassava Residue by Microbial Hydrolysis Using Bacteria Isolated from Thai Higher Termites. <u>Proceedings of The 3<sup>rd</sup> Research Symposium on</u> <u>Petroleum, Petrochemicals, and Advanced Materials and The 18<sup>th</sup> PPC Symposium</u> <u>on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.</u>

