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APPENDICES

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX A

1. Reagent preparation

1.1 Lactose broth (LB)

Beef Extract	3.0 g
Pancreatic Digest of Gelatin	5.0 g
Lactose	5.0 g
Water	1000 mL

Cool as quickly as possible after sterilization. pH after sterilization 6.7-6.9

1.2 Sauborand dextrose agar (SDA)

Dextrose	40.0 g
Mixture of equal parts of peptic digest of animal tissue and pancreatic digest of casein	10.0 g
Agar	15.0 g
Water	1000 mL

Mix, and boil to effect solution. pH after sterilization 5.4-5.8

1.3 Reagent for preparation McIlvaine buffer

Solution A: Citric acid 0.5 mol/L (citric acid monohydrate 105.07 g/L)

Solution B: di-sodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ 178.00 g/L)

The solution was Prepared by x part A + (100-x) part B in 100 mL,

Figure A1 Reagent for preparation McIlvaine buffer

pH	X part A	(100-X) part B
3.0	80.3	19.7
4.0	62.0	38.0
5.0	49.0	51.0
6.0	36.8	63.2
7.0	17.6	82.4
7.8	4.6	95.4

Adjust pH with 1 N NaOH

2. The calibration line for HPLC analysis of *N*-acetyl-D-glucosamine.

Table A2. Peak areas of GlcNAc standard for the calibration line Figure A1.

Standard No.	Conc. GlcNAc (mg/mL)	Conc. GlcNAc (mM)	Peak Area (mV*Sec)
1	0.0093	0.0420	14.983
2	0.0186	0.0841	27.667
3	0.0620	0.2803	98.046
4	0.1860	0.8408	308.345
5	0.1210	0.5470	195.536
6	0.2420	1.0940	387.937
7	0.4840	2.1880	749.393
8	1.2100	5.6055	1987.448

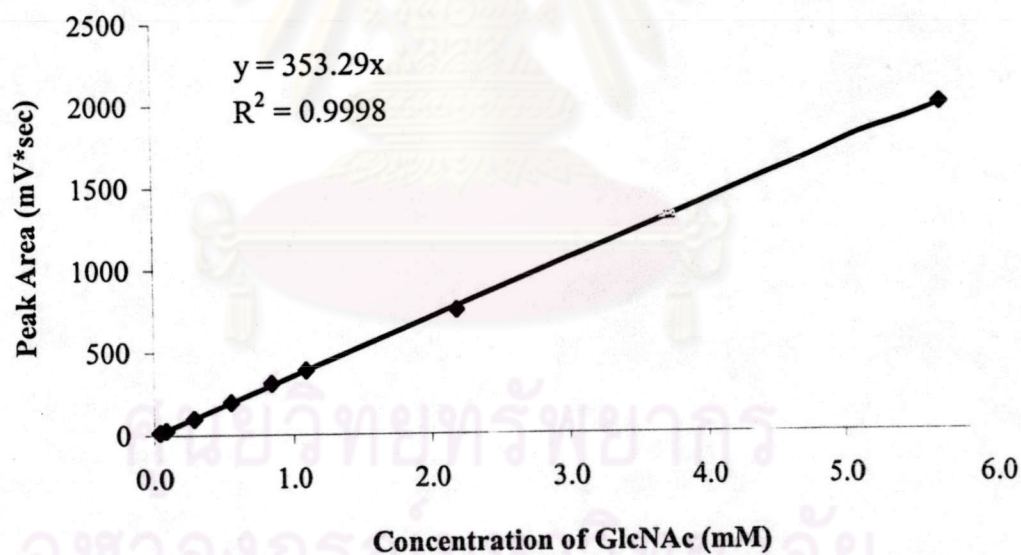


Figure A2 The calibration line for HPLC analysis of *N*-acetyl-D-glucosamine

3. The calibration line for HPLC analysis of *N,N'*-diacetylchitobiose .

Table A2 Peak areas of (GlcNAc)₂ standard for the calibration line A2

Standard No.	Conc. GlcNAc (mg/mL)	Conc. GlcNAc (mM)	Peak Area (mV*Sec)
1	0.05	0.1178	57.786
2	0.10	0.2356	111.022
3	0.20	0.4712	235.395
4	0.32	0.7540	380.000
5	0.60	1.4137	676.788
6	1.00	2.3562	1128.094
7	1.28	3.0159	1494.989
8	1.60	3.7699	1900.128

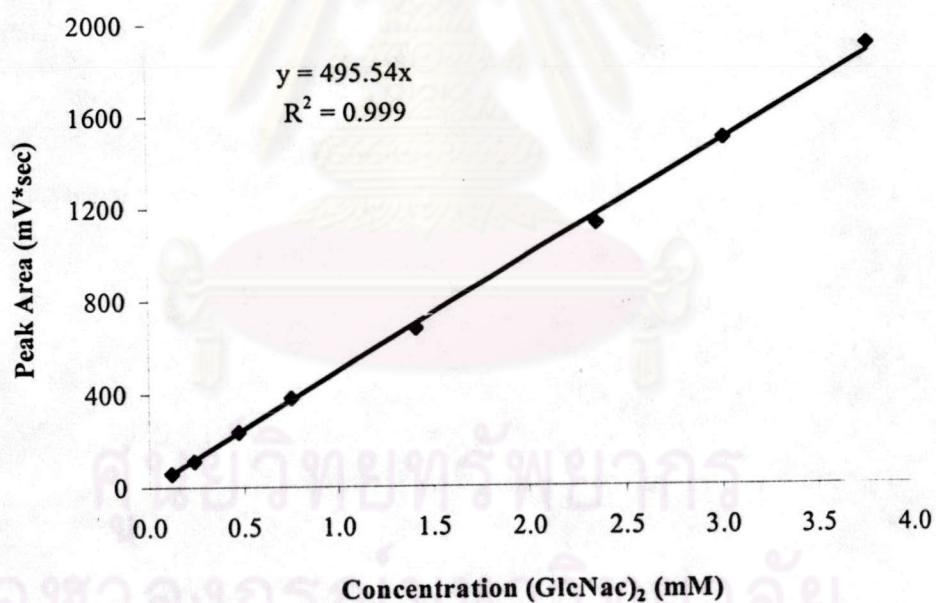


Figure A2 The calibration line for HPLC analysis of *N,N'*-diacetylchitobiose

4. The calibration line for HPLC analysis of *N,N',N''*-triacetylchitotriose.

Table A3 Peak areas of (GlcNAc)₃ standard for the calibration line Figure A3.

Standard No.	Conc. (GlcNAc) ₃ (mg/mL)	Conc. (GlcNAc) ₃ (mM)	Peak Area (mV*Sec)
1	0.050	0.080	64.77
2	0.200	0.319	281.76
3	0.400	0.637	557.62
4	0.600	0.956	827.87
5	0.800	1.275	1074.04
6	1.000	1.593	1376.31
7	1.200	1.912	1566.90
8	1.600	2.549	2081.22
9	2.000	3.187	2668.63

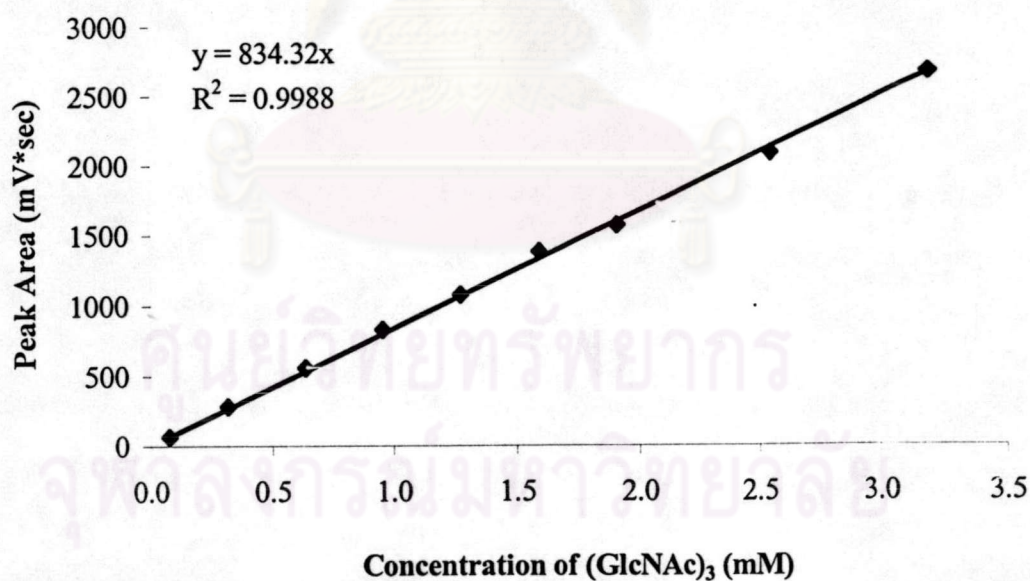


Figure A3 The calibration line for HPLC analysis of *N,N',N''*-triacetylchitotriose

5. The calibration line for enzyme assaying using GlcNAc as a standard table

Table A4 The Δ absorbance used for plotting the calibration line in Figure A4.

Standard No.	Amount of GlcNAc $\mu\text{mole/mL}$	Δ Absorbance
1	0.4123	0.798
2	0.3436	0.677
3	0.3526	0.703
4	0.2821	0.555
5	0.2116	0.429
6	0.1410	0.268
7	0.0705	0.133

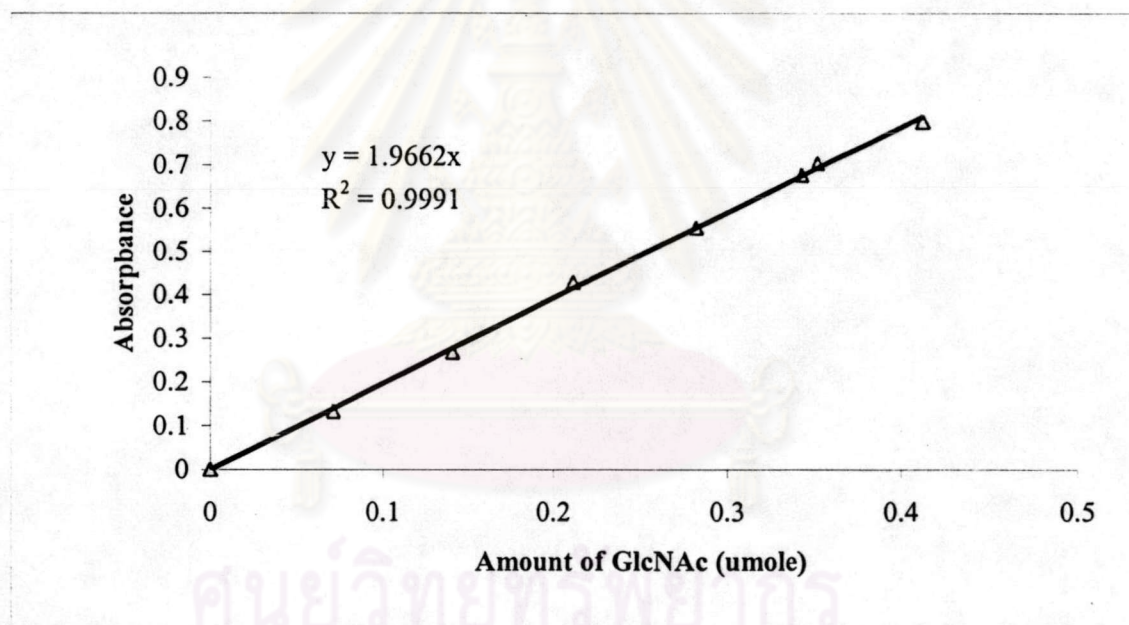


Figure A4 The calibration line for enzyme assaying using GlcNAc as a standard.

6. The calibration line for protein assaying by Biuret's colorimetric method.

Table A5 The absorbance of standard ovalbumine used for plotting the calibration line in Figure A5

Standard No.	Concentration of protein (mg/mL)	Absorbance
1	2.00	0.129
2	3.00	0.185
3	4.00	0.249
4	5.00	0.311
5	6.00	0.370
6	7.00	0.430

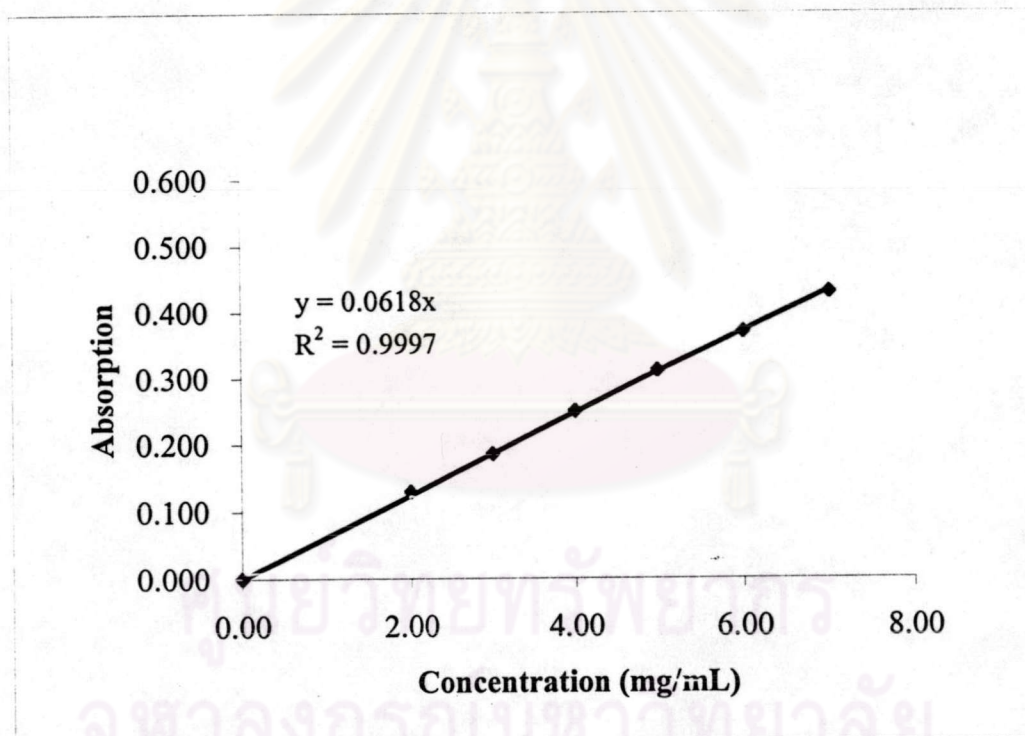


Figure A5 The calibration line for protein assaying by Biuret's colorimetric method.

APPENDIX B

Table B1 Numerical data for Figure 3.1

Type of carbon source	Lactose broth	Phosphate buffer	Beta-chitin	colloidal chitin
Type of fungi	(U/mL)	(U/mL)	(U/mL)	(U/mL)
<i>Aspergillus fumigatus</i>	0.007	0.007	0.019	0.125
<i>Trichoderma viride</i>	0.007	0.005	0.018	0.078
<i>Trichoderma aureviride</i>	0.004	0.005	0.011	0.014

Table B2 Numerical data for Figure 3.2

Type of nitrogen source	NH ₂ CONH ₂	(NH ₄) ₂ SO ₄	NH ₄ NO ₃
Type of fungi	(U/mL)	(U/mL)	(U/mL)
<i>Aspergillus fumigatus</i>	0.125	0.067	0.081
<i>Trichoderma viride</i>	0.095	0.060	0.100
<i>Trichoderma aureviride</i>	0.027	0.012	0.011
<i>Trichoderma reesei</i>	0.058	0.016	0.002
<i>Mucor sp.</i>	0.015	0.066	0.054

Table B3 Numerical data for Figure 3.3

Days	0	3	5	7	9	12	15
Chitinolytic Activity (U/mL)	0.00	0.05	0.081	0.104	0.118	0.118	0.111

Table B4 Numerical data for Figure 3.4

Number of inoculum (pieces)	0	3	5	7	10	14
Activity U/ml	0.00	0.040	0.073	0.102	0.061	0.067

Table B5 Numerical data for Figure 3.5

Days	Chitinolytic activity U/mL				
	0.00	5	7	9	12
None trace elements	0.00	0.081	0.104	0.118	0.118
Trace elements	0.00	0.126	0.130	0.136	0.134

Table B6 Numerical data for Figure 3.6

Days	Chitinolytic activity U/mL				
	0	5	7	9	12
Colloidal chitin 0.1 %	0.00	0.037	0.058	0.068	0.062
Colloidal chitin 0.3 %	0.00	0.095	0.099	0.11	0.112
Colloidal chitin 0.5 %	0.00	0.140	0.194	0.205	0.186
Colloidal chitin 1%	0.00	0.176	0.227	0.275	0.265
Colloidal chitin 2%	0.00	0.199	0.245	0.278	0.274
Colloidal chitin 3 %	0.00	0.197	0.241	0.281	0.277

Table B7 Numerical data for Figure 3.7

Days	Chitinolytic activity U/mL				
	0	5	7	9	12
Colloidal chitin 1%	0.00	0.195	0.242	0.285	0.274
Colloidal chitin 2%	0.00	0.188	0.245	0.275	0.271
Colloidal chitin 3 %	0.00	0.180	0.234	0.276	0.283

Table B8 Numerical data for Figure 3.8

Temperature °C	Days	Chitinolytic activity U/mL				
		0	5	7	9	12
30		0.00	0.176	0.227	0.275	0.265
40		0.00	0.345	0.409	0.438	0.423
50		0.00	0.000	0.000	0.000	0.000

Table B9 Numerical data for Figure 3.9

Type of buffer	MacIlvain/buffer							No buffer
pH	2.2	3.0	4.0	5.0	6.0	7.0	7.8	6.5
Relative activity (%)	22.6	100.0	94.8	90.0	75.7	71.2	60.6	85.3

Table B10 Numerical data for Figure 3.10

Temperature °C	30	37	45	55
Relative activity (%)	71.67	94.58	100.00	67.08

Table B11 Numerical data for Figure 3.11

Type of substrate	α -chitin	β -chitin	Colloidal chitin
Relative activity (%)	19.5	100.0	60.3

Table B12 Numerical data for Figure 3.12

Amount of enzyme (mU/mg)	0.0	0.5	1.0	2.0	3.0	4.0	5.0	5.5
Concentration μ mole/mL	0.0	22.4	37.1	61.7	69.3	79.6	87.0	87.3

Table B13 Numerical data for Figure 3.13

Concentration of buffer (M)	0.0	0.05	0.1	0.2
GlcNAc ($\mu\text{mole/mL}$)	71.7	72.8	76.1	70.27

Table B14 Numerical data for Figure 3.14

Substrate (mg/mL)	0	10	20	30	40	50	60
GlcNAc ($\mu\text{mole/ml}$)	0	23.0	62.0	73.6	92.6	94.7	87.2
(GlcNAc) ₂ ($\mu\text{mole/mL}$)	0	0.00	6.1	6.9	11.7	21.0	24.6
% yield GlcNAc	0	50.9	68.6	54.2	51.2	41.9	32.2
% yield (GlcNAc) ₂	0	0.0	12.9	9.76	12.4	17.8	17.4

Table B15 Numerical data for Figure 3.15

Days	GlcNAc (mg/mL)			
	0	1	3	5
None fed-batch (40 mg/mL)	21.2	-	28.6	21.2
Fed-batch	16.1	21.16	21.2	16.1
None fed-batch (20 mg/mL)	15.10	15.50	16.00	15.10

Table B16 Numerical data for Figure 3.16

Days	GlcNAc(%)					
	0	1	2	3	5	7
NaN ₃	0	74.12	50.55	54.98	45.72	51.65
Toluene	0	70.67	58.97	69.37	49.16	54.62

Table B17 Numerical data for Figure 3.17

Days	GlcNAc(%)					
	0	1	2	3	5	7
Fibrous chitin	0	70.67	58.97	69.37	49.16	54.62
Swollen chitin	0	58.59	48.14	56.20	58.47	54.45

VITAE

Miss. Krissana Auynirundronkul was born on May 3rd, 1971 in Phichit ,Thailand. Since 2001, she has been a graduate student studying petrochemistry and polymer science as her major course at Chulalongkorn University. During she studies towards the Master's degree, she was worked at Pfizer Company.

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