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APPENDICES

APPENDIX A

1. Reagent preparation

1.1 Reagent for protein assaying

- Bradford Stock solution

  100 mL 95% ethanol

  200 mL 85% phosphoric acid

  350 mg Serva Blue G

  stable indefinitely at room temperature.

- Bradford working Buffer

  425 mL distilled water

  15 mL 95% ethanol

  30 mL 85% phosphoric acid

  30 mL Bradford Stock Solution

  Filter through Whatman No. 1 paper, store at room temperature in brown glass bottle. Usable for several weeks, but may need to be refiltered.

1.2 Reagent for chitinase activity assaying

- Color reagent

Potassium hexaferrocyanate ($K_3Fe(CN_6)$) (0.5 g) and Na_2CO_3 (45.34 g) were dissolved in DI-water 500 mL.

2. Preparation the calibration curve of *N*-acetyl-D-glucosamine for HPLC analysis

Calibration curve of GlcNAc was made by varying the concentration and measuring the peak area by HPLC.

Table A1 The concentration of standard solution of GlcNAc and peak area.

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	1.2400	5.6055	1987.448

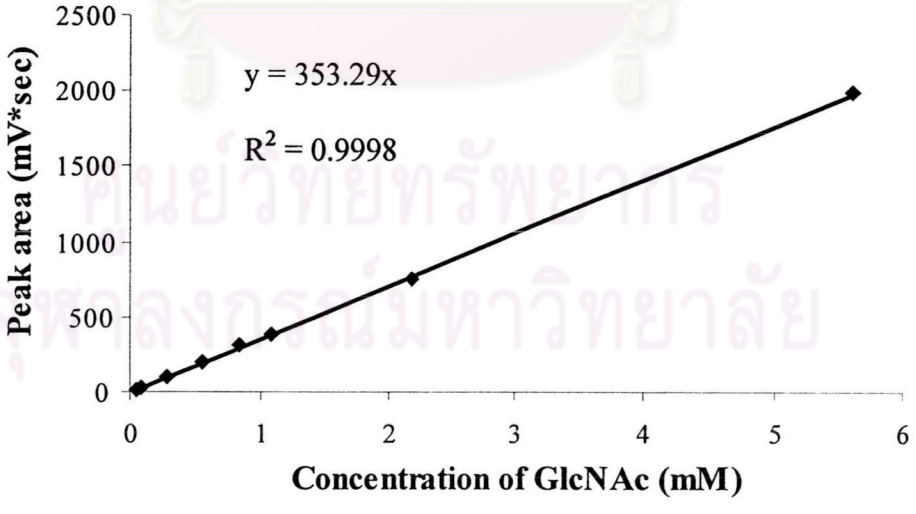

Figure A1 Correlation between concentration of standard *N*-acetyl-D-glucosamine and peak area by HPLC.

3. Preparation the calibration curve of *N,N'*-diacetylchitobiose for HPLC analysis

Calibration curve of $(GlcNAc)_2$ was made by varying the concentration and measuring the peak area by HPLC.

Table A2 The concentration of standard solution of $(GlcNAc)_2$ and peak area.

Standard No.	Conc. $(GlcNAc)_2$ (mg/mL)	Conc. $(GlcNAc)_2$ (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

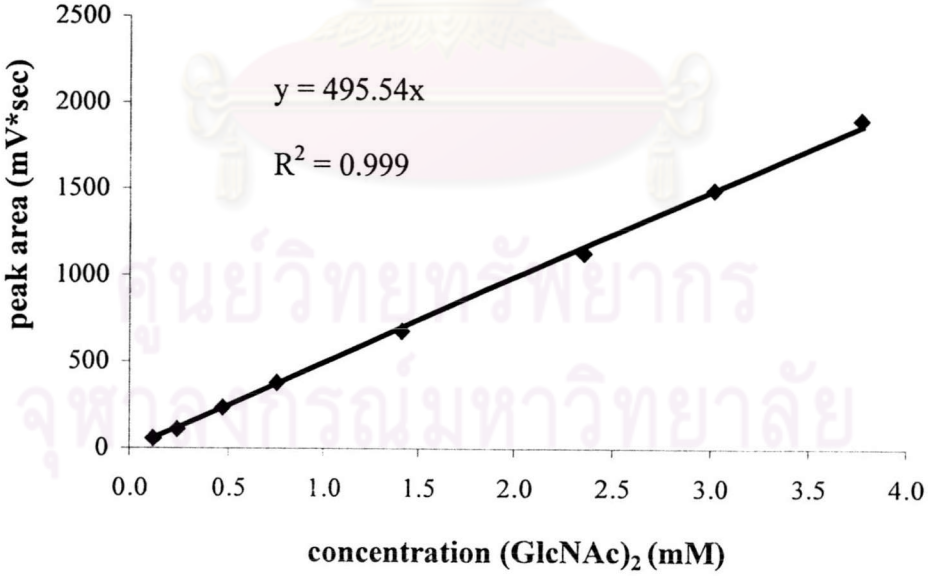

Figure A2 Correlation between concentration of standard *N,N'*-diacetylchitobiose and peak area by HPLC.

4. Preparation calibration curve of protein concentration by Bradford's colorimetric method

Calibration curve for BSA was made by determining the absorbance value at 595 nm of standard BSA according to the method of Bradford.

Table A3 The concentration of standard BSA and absorbance

Standard No.	μg BSA	Absorbance
1	0.0	0.000
2	2.5	0.097
3	5.0	0.150
4	7.5	0.208
5	10.0	0.249
6	12.5	0.291
7	15.0	0.367
8	17.5	0.379
9	20.0	0.427

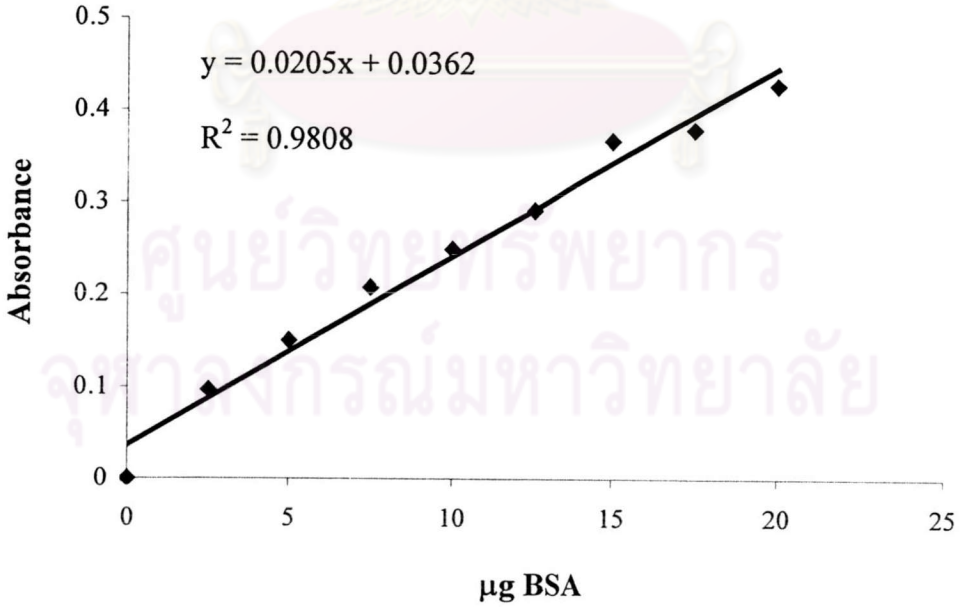

Figure A3 Relationship between standard protein (BSA) concentration and optical density (absorbance) at 595 nm.

5. Preparation calibration curve of N-acetyl-D-glucosamine for chitinolytic enzyme assay by colorimetric method

Calibration curve for GlcNAc was made by determining the absorbance value at 420 nm of standard GlcNAc according to the method of Schales.

Table A4 The amount of standard solution of GlcNAc and ΔAbsorbance

Standard No.	Amount of GlcNAc (μmole)	ΔAbsorbance
1	0.8029	0.886
2	0.7025	0.761
3	0.6021	0.650
4	0.5018	0.541
5	0.5018	0.516
6	0.4014	0.411
7	0.3011	0.311
8	0.2007	0.187
9	0.1004	0.094

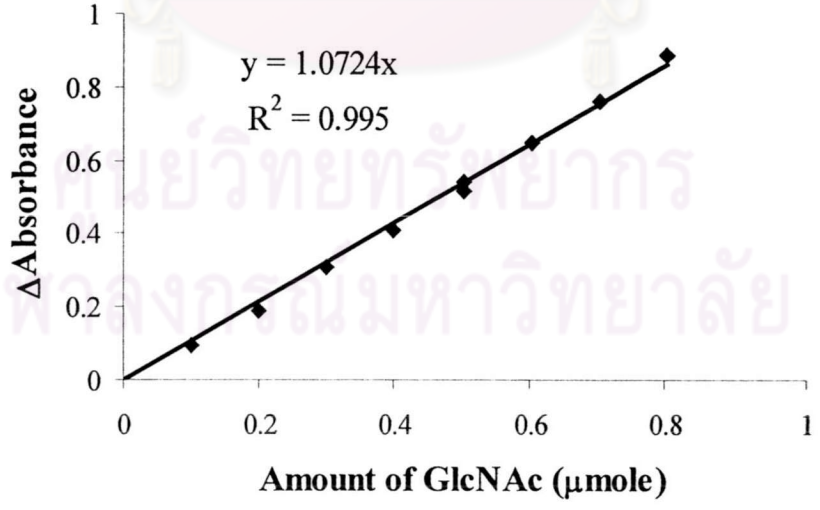

Figure A4 Correlation between amount of standard of *N*-acetyl-D-glucosamine and optical dencity (absorbance) at 420 nm.

APPENDIX B

Table B1 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of powder chitin on chitinolysis (The data in bold fonts were used in Figure 3.2).

[chitin] (mg/mL)	Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
10.0	1	3.32	4.52	1.36
	2	**6.06**	**6.98**	**1.15**
	3	7.82	7.84	1.00
	4	10.23	8.85	0.87
20.0	1	3.35	6.84	2.04
	2	**5.59**	**9.60**	**1.72**
	3	7.39	11.02	1.49
	4	9.01	12.02	1.33
30.0	1	2.87	7.42	2.58
	2	**4.95**	**10.93**	**2.21**
	3	6.73	12.86	1.91
	4	8.34	14.64	1.76
40.0	1	2.83	7.80	2.76
	2	**4.64**	**11.08**	**2.39**
	3	8.11	14.90	1.84
	4	8.75	15.44	1.76
50.0	1	2.66	8.06	3.03
	2	**4.24**	**11.28**	**2.66**
	3	7.55	15.62	2.07
	4	9.0	17.88	1.99
60.0	1	2.38	7.80	3.27
	2	**3.92**	**11.22**	**2.86**
	3	7.01	15.54	2.22
	4	7.98	17.01	2.13
80.0	1	1.19	5.02	4.22
	2	**3.56**	**10.95**	**3.07**
	3	5.14	14.55	2.83
	4	6.10	16.49	2.70

Table B1 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of powder chitin on chitinolysis (continued).

[chitin] (mg/mL)	Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
100.0	1	1.48	5.19	3.52
	2	**2.46**	**7.72**	**3.14**
	3	3.80	11.05	2.90
	4	4.85	13.67	2.82
200.0	1	0.90	3.06	3.39
	2	**1.37**	**4.79**	**3.49**

Table B2 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of fibrous chitin on chitinolysis (The data in bold fonts were used in Figure 3.3).

[chitin] (mg/mL)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
10.0	1	3.39	3.53	1.04
	2	**5.49**	**4.31**	**0.79**
	3	7.55	4.68	0.62
	5	11.04	4.50	0.41
20.0	1	3.13	3.94	1.26
	2	**5.01**	**5.96**	**1.19**
	3	7.04	7.37	1.05
	5	10.25	8.69	0.85
30.0	1	3.28	5.60	1.71
	2	**4.79**	**7.76**	**1.62**
	3	6.57	9.62	1.46
	5	9.06	10.79	1.19
60.0	1	2.68	5.81	2.17
	2	**3.98**	**8.99**	**2.26**
	3	5.25	10.89	2.07
	5	8.92	15.97	1.79

Table B3 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of cellulase *Ac* on chitinolysis (The data in bold fonts were used in Figure 3.4).

[cellulase *Ac*] (mU/mL)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
130.0	1	1.09	3.86	3.54
	3	3.09	10.01	3.24
	5	**3.89**	**12.12**	**3.12**
	9	5.22	14.43	2.76
520.0	1	4.68	10.56	2.26
	3	12.06	21.54	1.79
	5	**17.96**	**29.66**	**1.65**
	9	30.84	40.04	1.30
780.0	1	7.02	14.53	2.07
	3	17.48	29.04	1.66
	5	**26.99**	**40.21**	**1.49**
	9	44.93	49.40	1.10
1040.0	1	9.11	15.98	1.75
	3	21.86	30.37	1.39
	5	**30.49**	**38.41**	**1.26**
	9	52.16	47.48	0.91

Table B4 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of cellulase *Ac* on chitinolysis (The data in bold fonts were used in Figure 3.5).

[cellulase *Ac*] (mU/mL)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
159.5	1	2.83	5.64	1.99
	3	5.58	8.92	1.60
	5	**10.42**	**16.48**	**1.58**
	9	7.94	11.92	1.50
319.0	1	4.98	8.71	1.75
	3	9.20	13.64	1.48
	5	**19.06**	**28.24**	**1.48**
	9	17.02	23.16	1.36
638.0	1	7.70	11.26	1.46
	3	18.30	21.14	1.16
	5	**33.12**	**36.12**	**1.09**
	9	28.62	26.64	0.93
957.0	1	10.41	13.81	1.33
	3	24.34	24.60	1.01
	5	**51.74**	**48.72**	**0.94**
	9	45.54	36.30	0.80
1276.0	1	12.55	17.17	1.37
	3	31.64	30.96	0.98
	5	**47.80**	**42.10**	**0.88**
	9	55.44	38.80	0.70

Table B5 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of pH of the reaction solution on chitinolysis (The data in bold fonts were used in Figure 3.6).

McIlvaine buffer (pH)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
2.0	1	2.70	2.52	0.93
	3	**6.96**	**5.56**	**0.80**
	5	19.98	14.30	0.72
3.0	1	9.11	13.82	1.52
	3	**17.74**	**21.91**	**1.24**
	5	27.76	31.96	1.15
4.0	1	9.13	12.08	1.32
	3	**16.45**	**17.17**	**1.04**
	5	35.97	31.43	0.87
5.0	1	6.01	7.61	1.27
	3	**12.47**	**11.83**	**0.95**
	5	20.66	18.25	0.88

Table B6 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of buffer on chitinolysis (The data in bold fonts were used in Figure 3.7).

[McIlvaine buffer] (M)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
0.0	1	1.96	0.82	0.42
	3	**1.29**	**1.37**	**1.06**
	9	3.30	3.90	1.18
0.05	1	6.49	11.40	1.76
	3	**13.56**	**18.29**	**1.35**
	5	15.61	23.64	1.51
	9	33.81	30.60	0.91
0.1	1	8.25	15.61	1.89
	3	**15.70**	**24.62**	**1.57**
	5	24.75	32.78	1.32
	9	39.49	38.00	0.96
0.2	1	8.42	18.57	2.21
	3	**19.90**	**31.36**	**1.58**
	5	24.55	37.71	1.54
	9	37.93	42.10	1.11
0.4	1	6.42	15.97	2.49
	3	**14.74**	**27.80**	**1.89**
	9	26.65	36.20	1.36

Table B7 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of temperature on chitinolysis (The data in bold fonts were used in Figure 3.8).

Temperature (°C)	Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
30	1	7.52	15.47	2.06
	3	16.72	27.42	1.64
	5	**22.85**	**32.35**	**1.42**
	9	33.23	45.26	1.36
37	1	7.02	14.53	2.07
	3	17.48	29.04	1.66
	5	**26.99**	**40.21**	**1.49**
	9	44.93	49.40	1.10
45	1	11.81	17.95	1.52
	3	23.86	32.02	1.34
	5	**32.62**	**37.89**	**1.16**
	9	44.87	44.11	0.98
55	1	2.14	7.31	3.42
	3	16.67	5.50	0.33
	5	**22.42**	**4.54**	**0.20**

Table B8 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of enzyme affinity technique on chitinolysis (The data in bold fonts were used in Figure 3.9).

Chilling period (hrs)	Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
0	1	1.18	5.37	4.57
	2	2.21	8.23	3.73
	3	**2.74**	**9.64**	**3.52**
	4	3.28	10.87	3.31
1	1	1.36	5.60	4.11
	2	2.32	8.08	3.49
	3	**3.00**	**9.82**	**3.27**
	4	3.48	10.79	3.10
4	1	0.92	4.57	4.99
	2	1.58	6.59	4.16
	3	**2.31**	**8.90**	**3.85**
	4	2.78	10.20	3.67
24	1	1.18	5.37	4.55
	2	2.09	8.15	3.90
	3	**2.65**	**9.71**	**3.66**
	4	3.19	11.08	3.47

Table B9 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of fibrous chitin on chitinolysis (The data in bold fonts were used in Figure 3.10).

[chitin] (mg/mL)	Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
10.0	1	0.16	4.67	28.84
	2	0.35	6.63	19.04
	3	0.57	8.06	14.19
	4	0.61	8.97	14.68
	7	**0.79**	**9.73**	**12.27**
20.0	1	0.12	5.24	44.42
	2	0.26	7.25	28.09
	3	0.43	9.28	21.74
	4	0.57	10.57	18.64
	7	**0.76**	**11.61**	**15.33**
30.0	1	trace	3.17	-
	2	trace	6.17	-
	3	trace	8.66	-
	4	0.18	7.92	43.75
	7	**0.42**	**10.67**	**25.64**
40.0	6	0.25	10.06	40.89
	7	**0.25**	**7.82**	**31.41**

Table B10 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of Chi 60 on chitinolysis (The data in bold fonts were used in Figure 3.11).

[cellulase *Ac*] (mU/mL)	Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
4.0	1	Not observed	4.35	-
	2	Not observed	**5.04**	-
	3	Not observed	5.14	-
	6	Not observed	6.41	-
	8	Not observed	6.48	-
5.0	1	Not observed	5.30	-
	2	Not observed	**6.97**	-
	3	Not observed	7.05	-
	6	Not observed	9.06	-
	8	Not observed	9.08	-
6.55	1	Not observed	6.37	-
	2	Not observed	**8.46**	-
	3	Not observed	9.20	-
	6	Not observed	11.29	-
	8	Not observed	11.07	-
9.32	1	Not observed	7.70	-
	2	Not observed	**9.95**	-
	3	Not observed	10.63	-
	6	Not observed	14.61	-
	8	Not observed	17.10	-
11.0	1	Trace	9.22	-
	2	Trace	**12.29**	-
	3	Trace	13.14	-
	6	trace	16.98	-
	8	Trace	18.44	-

Table B10 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of Chi 60 on chitinolysis (continued).

[cellulase *Ac*] (mU/mL)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
13.0	1	Trace	10.24	-
	2	Trace	**12.59**	-
	3	Trace	14.26	-
	6	Trace	19.72	-
	8	Trace	19.79	-
15.0	1	Trace	11.77	-
	2	Trace	**14.39**	-
	3	Trace	15.34	-
	6	Trace	22.19	-
	8	Trace	22.11	-
30.0	1	0.97	15.34	15.81
	2	**1.41**	**19.21**	**13.62**
	3	1.58	21.33	13.50
	6	1.89	23.60	12.49
	8	1.97	23.74	12.05
50.0	1	1.80	20.53	11.41
	2	**2.54**	**24.99**	**9.84**
	3	2.93	27.04	9.23
	6	3.61	31.53	8.73
	8	3.65	32.48	8.90
100.0	1	3.85	27.02	7.02
	2	**5.32**	**32.86**	**6.18**
	3	5.84	34.95	5.98
	6	6.29	37.99	6.04
	8	6.13	36.94	6.03

Table B10 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of Chi 60 on chitinolysis (continued).

[cellulase *Ac*] (mU/mL)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
150.0	1	9.31	26.66	2.86
	2	**17.72**	**38.88**	**2.19**
	3	20.46	41.30	2.02
	6	26.32	46.40	1.76
	8	26.60	44.98	1.69
250.0	1	7.82	38.26	4.89
	2	**17.25**	**41.05**	**2.38**
	3	17.36	40.68	2.34
	6	21.56	42.10	1.95
	8	21.70	41.50	1.91
350.0	1	15.12	36.64	2.42
	2	**20.85**	**42.40**	**2.03**
	3	20.40	40.00	1.96
	6	29.46	39.52	1.34
	8	34.90	38.88	1.11

Table B11 The yield of $(GlcNAc)_2$ by the effect of pH of the reaction solution on chitinolysis (The data in bold fonts were used in Figure 3.12).

McIlvaine buffer (pH)	Time (day)	$[(GlcNAc)_2]$ (mM)
3.0	1	0.10
	2	0.11
	3	**0.13**
	4	0.14
4.0	1	0.29
	2	0.26
	3	**0.27**
	4	0.28
5.0	1	0.88
	2	0.98
	3	**1.16**
	4	1.31
6.0	1	1.65
	2	2.03
	3	**2.21**
	4	2.40
7.0	1	0.93
	2	1.43
	3	**1.70**
	4	2.05

Table B12 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of buffer on chitinolysis (The data in bold fonts were used in Figure 3.13).

[McIlvaine buffer] (M)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
0.0	3	1.75	14.58	8.33
	5	4.93	34.94	7.09
	9	7.42	40.93	5.52
0.05	**3**	**2.19**	**15.34**	**7.00**
	5	5.46	34.39	6.30
	9	8.05	40.06	4.98
0.1	**3**	**2.33**	**15.25**	**6.55**
	5	5.93	34.45	5.81
	9	8.59	40.25	4.69
0.2	**3**	**1.93**	**13.64**	**7.07**
	5	5.01	31.32	6.25
	9	6.87	35.89	5.22
0.4	**3**	**1.69**	**10.31**	**6.10**
	5	-	26.00	-
	9	-	23.59	-

Table B13 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of temperature on chitinolysis (The data in bold fonts were used in Figure 3.14).

Temperature (°C)	Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
32	1	8.26	28.33	3.43
	2	12.48	35.00	2.80
	3	**13.63**	**37.52**	**2.75**
	6	15.86	41.82	2.64
	8	15.68	36.16	2.31
37	1	9.31	26.66	2.86
	2	17.72	38.88	2.19
	3	**20.46**	**41.30**	**2.02**
	6	26.32	46.40	1.76
	8	26.60	44.98	1.69
45	1	18.59	38.35	2.06
	2	23.38	38.72	1.66
	3	**25.20**	**39.12**	**1.55**
	6	29.98	40.18	1.34
	8	30.00	36.14	1.20
50	1	13.61	32.49	2.39
	2	11.68	33.92	2.90
	3	**18.56**	**36.56**	**1.97**
	6	19.16	37.60	1.96
	8	21.78	39.46	1.81

Table B14 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of enzyme affinity technique on chitinolysis (The data in bold fonts were used in Figure 3.15).

Chilling period (hrs)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
Non adsorption	1	9.31	26.66	2.86
	2	17.72	38.88	2.19
	4	20.46	41.30	2.02
	6	26.32	46.40	1.76
	8	**26.60**	**44.98**	**1.69**
0	1	0.95	8.12	8.55
	2	3.24	11.10	3.43
	4	3.63	17.04	4.69
	6	5.64	22.87	4.06
	8	**5.61**	**23.19**	**4.14**
1	1	0.93	7.59	8.16
	2	2.28	10.82	4.75
	4	2.31	16.78	7.26
	6	4.83	20.64	4.27
	8	**5.39**	**21.70**	**4.02**
3	1	0.85	7.04	8.28
	2	2.84	9.95	3.50
	4	4.46	16.30	3.65
	6	4.92	21.93	4.46
	8	**6.05**	**23.08**	**3.81**
24	1	0.74	11.32	15.30
	2	3.04	13.75	4.52
	4	4.76	20.06	4.22
	6	6.53	24.45	3.75
	8	**6.98**	**26.32**	**3.77**

VITAE

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