APPENDIX A

Reducing Sugar Determination by DNS Method (Watunyoo, 2003)

Chemical reagents

DNS (3,5-dinitrosalicylic acid)	10 g
Na_2SO_3	0.5 g
NaOH	16 g
Na-K tatrate	300 g
Phenol	2 g
Distilled water	1 L

DNS solution preparation:

1. Dissolve NaOH in 250 mLof distilled water.
2. Add DNS and stir continuously.
3. Add Na-K tatrate, stir ultill well dissolve.
4. Add Na_2SO_3 and phenol, respectly.
5. Adjust to final volume of 1 L with volume metric flask.
6. Keep DNS solution in brown glass bottle.

Reducing sugar determination procedure

1. Mix 1 mL of sample with 1 mL of DNS solution and boil for 10 minutes.
2. Cool down the sample by immerse the sample tube into cold water immediately, add 10 mL of distilled water, mix well, and measure A_{540}.
3. Convert A_{540} to reducing sugar concentration with standard curve.

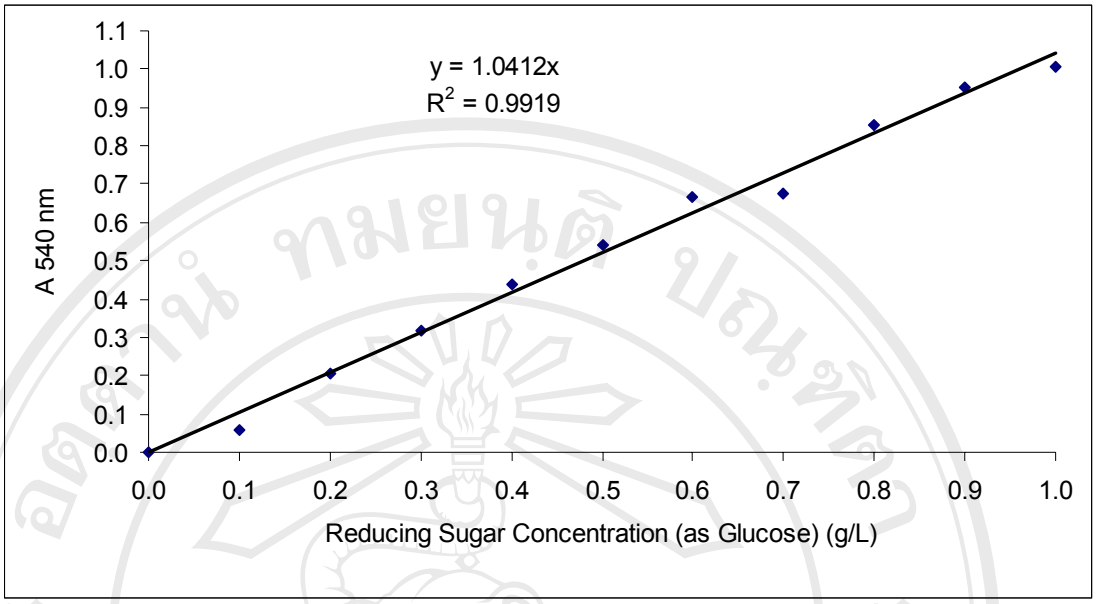

Figure A1 Standard Curve of Reducing Sugar (as Glucose).

APPENDIX B

Biomass Determination by Spectrophotometer at 550 nm (Watunyoo, 2003)

Standard curve preparation

1. *Saccharomyces cerevisiae* was cultured in 100 mL of YM broth, shaken at 180 rpm for 24 h.
2. All the cultured broth was centrifuged at 3,400 rpm for 15 minutes.
3. Supernatant was decanted, and cells were washed twice with deionized water.
4. The cell from 3 was diluted with deionized water, and the total volume was made up in 10 mL volumetric flask.
5. Five ml. of cell suspension from 4 was filtered through known-weight Millipore membrane, dried in the hot air oven at 105 ^{o}C for over night. Cell dried weight was calculated out in g/L.
6. Cell suspension from 4 was also serial diluted.
7. Measuring of cell absorbances at 550 nm from various cell suspensions obtained from 6.
8. Relationship between absorbances at 550 nm and cell dried weights were determined.
9. Standrad curve of biomass was shown in Figure B1.

Procedure

1. Zero the Spectrophotometer using distilled water.
2. Measure the absorbance of the sample. (using a 1 cm cuvette).
3. Record the absorbance and estimate of biomass by comparing with standard curve.

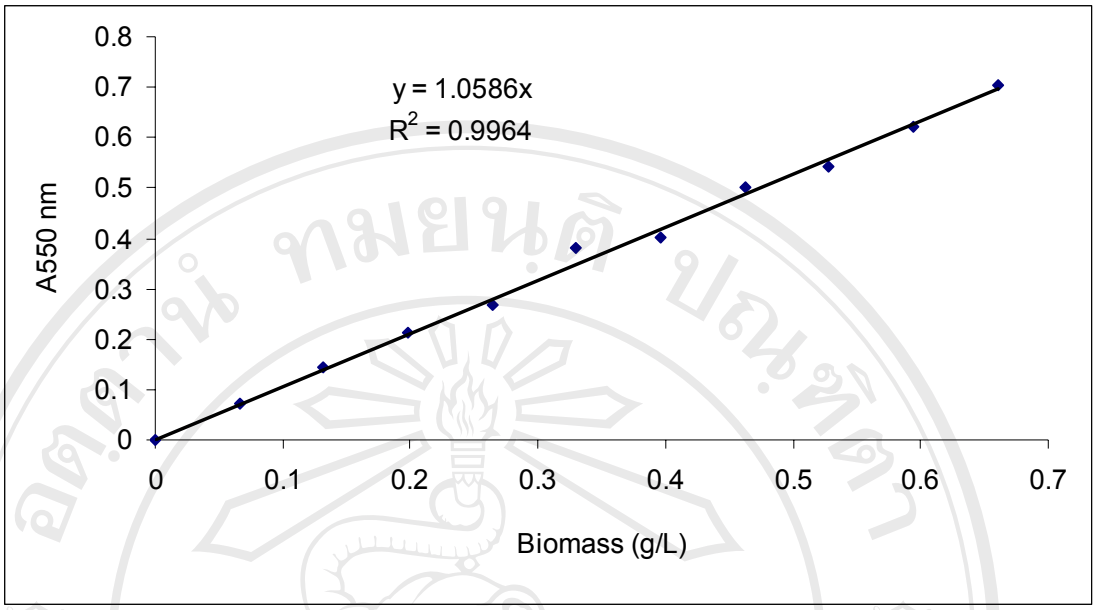

Figure B1 Standard Curve of Biomass.

CURRICULUM VITAE

Name: Mr. Witayapan Nantitanon

Date of Birth: September 4, 1979

Academic background: 1999 High school, Suanboonyopathum Lamphun School, Lamphun.

2002 B.S. (Biochemistry and Biochemistry Technology) Faculty of Science, Chiang Mai University.