Appendix A

Medium and solution preparation

A1) A mineral salt agar (Ralston and Vela, 1979)

The medium contained (g/l):

$NaHCO_3$	0.125	g
KH_2PO_4	0.1	g
NH_4Cl	0.07	g
Na_2SiO_3	0.02	g
$FeSO_4.7H_2O$	0.01	g
$MnCl_2.4H_2O$	0.007	g
$ZnSO_4.7H_2O$	0.0015	g
Casamino acid	0.01	g
Agar	15.00	g

Medium pH was adjusted to 8.0 with 1 N NaOH solution and was brought to boil to dissolve the agar completely. Aliqout the solution in 5 ml portions into vials and then sterilize by autoclaving at 121 ºC for 15 minutes. After autoclaving, phenol

was added to a concentration of 0.2 g/l and the vials were left to solidify in a slanted position.

A2) A mineral salt broth (Ralston and Vela, 1979)

The medium contained (g/l):

$NaHCO_3$	0.125	g
KH_2PO_4	0.1	g
NH_4Cl	0.07	g
Na_2SiO_3	0.02	g
$FeSO_4.7H_2O$	0.01	g
$MnCl_2.4H_2O$	0.007	g
$ZnSO_4.7H_2O$	0.0015	g
Casamino acid	0.01	g

Medium pH was adjusted to 8.0 with 1 N NaOH solution before sterilization at 121 ºC for 15 minutes. Filter sterilized phenol was added to the sterilized medium to a specificed concentration.

A3) Yeast malt broth

The medium contained (g/l):

peptone	5.0	g
yeast extract	3.0	g
malt extract	3.0	g
glucose	10.0	g
deionize water	1	L

The pH was adjusted to 8.0 with 1 N NaOH solution. Aliqout a specific volume into 500 ml Erlenmeyer flasks and sterilize at 121 ºC for 15 minutes.

A4) Stock phenol solution

Stock phenol solution was prepared by dissolving 12 g phenol flakes in deionized water and diluted to 1 L. This solution was added to the cultivation medium to a specified concentration.

Appendix B

Chemical analyses

B) Phenol analysis (Greenberg *et al*., 1992)

B 1.1) Reagents

Prepare all reagents with deionzed water free of phenol and chlorine.

a) Stock phenol solution: Dissolve 100 mg phenol in freshly boiled and cooled deionized water and dilute to 100 ml.

b) Intermediate phenol solution: Dilute 1.00 ml stock phenol solution in freshly boiled and cooled deionized water to 100 ml (1 ml = 10 µg phenol). Prepare daily.

c) Standard phenol solution: Dilute 50.0 ml intermediate phenol solution to 500 ml with freshly boiled and cooled deionized water (1 ml = 1.0 µg phenol). Prepare within 2 hours of use.

d) Ammonium hydroxide (NH_4OH) 0.5 N: Dilute 35 ml fresh, conc. NH_4OH to 1 L with deionzied water.

e) Phosphate buffer solution: Dissolve 104.5 g K_2HPO_4 and 72.3 g KH_2PO_4 in deionized water and dilute to 1 L. The pH should be 6.8.

f) 4-aminoantipyrine solution: Dissolve 2.0 g 4-aminoantipyrine in deionized water and dilute to 100 ml. Prepare daily.

g) Potassium ferricyanide solution: Dissolve 8.0 g $K_3Fe(CN)_6$ in deionzed water and dilute to 100 ml. Filter if necessary. Store in a brown glass bottle. Prepare fresh weekly.

B 1.2) Apparatus

a) a spectrophotometer (Shimadzu 1601, Japan)

b) a pH meter (Cyberscan 500, Eutech Instruments)

B 1.3) Procedure

1) Place a portion of sample containing not more than 0.5 mg phenol dilute to 100 ml, in a 250 ml beaker.

2) Prepare a 100 ml deionzed water blank and a series of 100 ml phenol standards containing 0.1, 0.2, 0.3, 0.4 and 0.5 mg phenol.

3) Treat sample, blank and standards as follows:

3.1) Add 2.5 ml 0.5N NH_4OH solution and immediately adjust to pH 7.9 ±0.1 with phosphate buffer

3.2) Add 1.0 ml 4-aminoantipyrine solution, mix well, add 1 ml $K_3Fe(CN)_6$ solution and mix well

3.3) After 15 minutes, transfer to a cell and read absorbance of samples and standards against the blank at 500 nm.

Construct a calibration curve by plotting absorbances against mg phenol concentrations.

Estimate sample phenol mg from spectrophotometric readings by using a calibration curve:

$$\text{mg phenol/l} = (A/B) \times 1000$$

where:

A = mg phenol in sample from a calibration curve

B = ml original sample

Appendix C

Calibration graphs

C1) Biomass

The relationship between cell dry weight and optical density of *Candida tropicalis* culture broth measured at 600 nm is illustrated in Figure C 1.

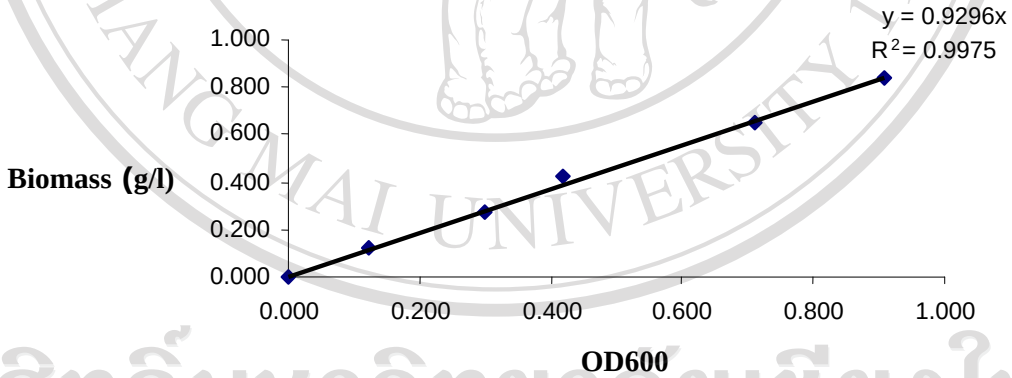

Figure C 1. Biomass calibration graph (suspended growth).

This , therefore, provides the equation below for the calculation of biomass concentration from the optical density of the culture at 600 nm.

Biomass concentration (g/l) = 0.9296 x optical density

C2) Phenol

The relationship between phenol concentration (g/l) and optical density of a sample measured at 500 nm is illustrated in Figure C 3.

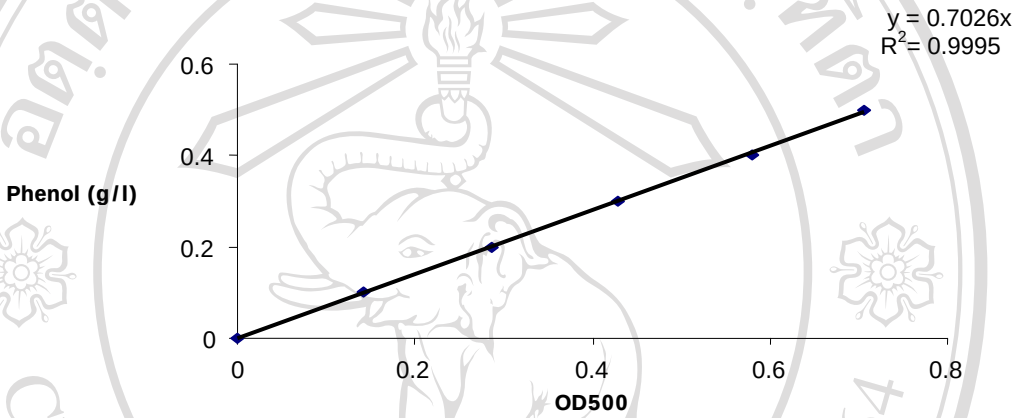

Figure C 2. Phenol calibration graph.

This, therefore, provides the equation below for the calculation of phenol concentration in a sample from the optical density at 500 nm.

Phenol concentration (g/l) = 0.7026 x optical density

CIRRICULUM VITAE

Name Miss Montira Intanon

Date of birth 5 September 1983

Academic background 2005 B.S. (Agro-Industrial Biotechnology)
Faculty of Agro-Industry, Chiang Mai University
Chiang Mai, Thailand

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