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APPENDIX

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APPENDIX 1

1. Protein Determination by Lowry method**1.1. Reagents**

Alkaline Copper reagent:

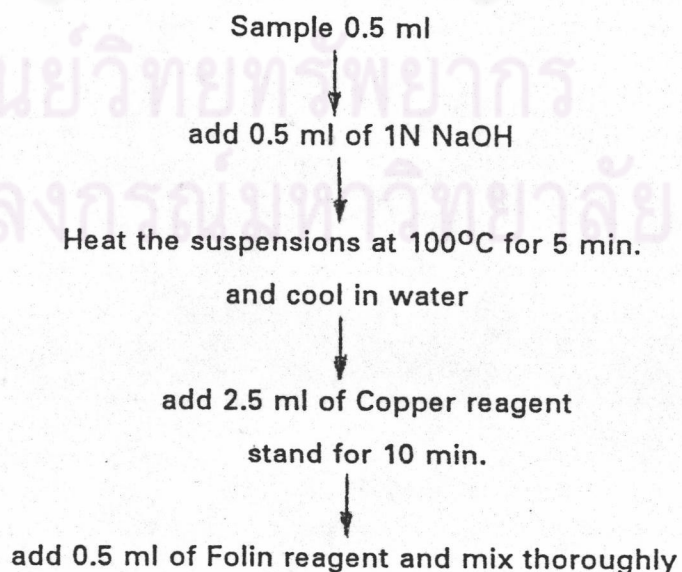
- A. Solution of 5 % Na_2CO_3 in distillation water.
 - B. Dissolve 0.5 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 100 ml of distillation water.
 - C. Aqueous solution. of sodium potassium tartrate 2 %
- Just before use, mix 50 ml of A, 1 ml of B and 1ml of C.
Discard after 1 day.

Folin reagent:

- Concentrated Folin-Ciocalteu reagent can be purchased commercially from SIGMA Chemical company and is diluted 1:1 with water.

(CAUTION : This reagent contains a strong volatile acid and should not be pipetted by mouth.)

- Solution of 1N NaOH

1.2. Procedure.

stand for at 30 min.



measure optically density (OD) at wave length 750 nm
in spectrophotometer 21

Standard protein solution: Crystalline bovine serum albumin is use for standard protein solution and linear standard curves. The stock standard solution should contain 0.2 mg of protein per ml in distilled water. Different proteins give different standard curves. The protein standard used should be specified when the results are reported.

2. Determination of sugars by Somogyi and Nelson method

2.1. Reagents

Somogyi reagent

Copper reagent A

Dissolve the following chemicals :

- Na ₂ CO ₃	12.5 g
- C ₄ H ₄ Na ₂ O ₈ .4H ₂ O	12.5 g
- NaHCO ₃	10 g
- Na ₂ SO ₄	100 g

in 500 ml of distillated water.

Copper reagent B

Dissolve the following chemicals :

- CuSO ₄ .5H ₂ O	15 g-
- H ₂ SO ₄ Conc.	1-2 drop

in 100 ml of distillated water.

- Just before use, mix 25 ml of A, 1 ml of B. Discard after 1 day.

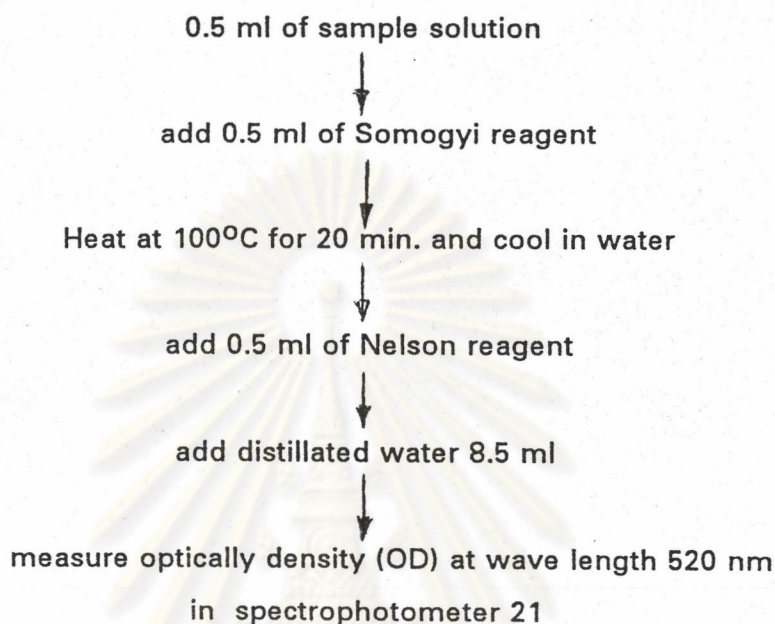
Nelson reagent

Dissolve the following chemicals :

- (NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	12.5 g
- H ₂ SO ₄ Conc.	10.5 ml
- Na ₂ HAsO ₄	1.5 g

in 250 ml of distilled water. Keep at 37°C for 48 hour and should appear yellow color.

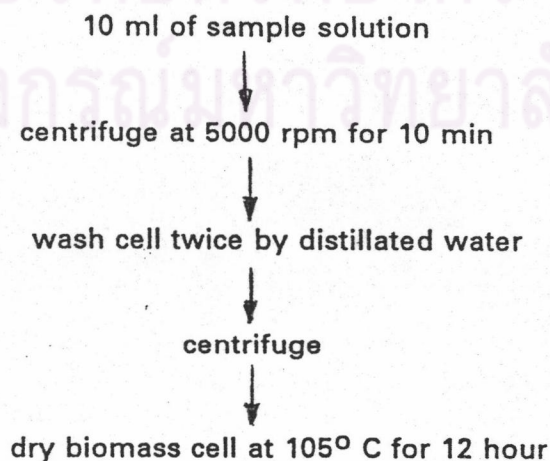
2.2. Procedure.



Standard glucose solution : pure glucose is used for standard protein solution and linear standard curves. The stock standard solution should contain 0.2 mg of glucose per ml in distilled water. Different glucose give different standard curves. The glucose standard used should be specified when the results are reported.

3. Determination of dry weight

3.1. Procedure





↓
weigh

4. Determination of starch in cassava chip :

4.1 Acid hydrolysis of carbohydrate (glucose car.)

1 g of sample into flask of 100 ml
(flask had a tube of glass for condensation)

↓
add 10 ml of 25 % HCL

↓
heat at 100°C in water bath for 3 h.

↓
cool to 30°C

↓
neutralize with 40 % NaOH

↓
dilute with distilled water to have glucose 5-100 ug/ml

↓
Determination of glucose car. by method of Somogyi and Nelson

4.2 Acid hydrolysis of reducing sugar (glucose red.)

1 g of sample into flask of 100 ml
(flask had a tube of glass for condensation)

↓
add 40 ml 0.1 % HCL

↓
heat at 100°C in water bath for 30 min

↓
cool to 30°C

↓
neutralize with 1 N. NaOH to pH 5.0

↓
dilute with distilled water to have glucose 5-100 ug/ml

↓
Determination of glucose red. by method of Somogyi and Nelson

4.3 Calculation of starch

$$\text{Starch} = (\text{glucose car.} - \text{glucose red.}) \times 0.9$$



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จุฬาลงกรณ์มหาวิทยาลัย

APPNDIX 2

4.1 Calculation of the Reynolds Number (Re)

$$Re = (\rho N D^2) / \mu$$

ρ = density of broth 3.5 % of sucrose at 30°C 1.077 g/cm³

μ = viscosity of broth 3.5 % of sucrose at pH 4-6, 30 °C
(4 X 10⁻² g/cm.sec)

N = agitator speed 140 rpm or min⁻¹

D = impeller diameter 20 cm

$$Re = \frac{(1.077 \text{ g/cm}) \times (140 \text{ min}^{-1}) \times (20 \text{ cm})^2}{(4 \times 10^{-2} \text{ g/cm.sec}) \times (60 \text{ sec/min.})}$$

$$= 25130$$

4.2 Calculation of kinetics parameter for *C. utilis*

U_{\max} : the maximum specific growth rate

$$U_{\max} = \frac{\ln(x_2) - \ln(x_1)}{t_2 - t_1} = \frac{1.29 - 0.29}{21 - 9} = 0.083 \text{ h}^{-1}$$

$Y_{x/s}$: the biomass yield

$$Y_{x/s} = \frac{m_x}{m_s} = \frac{4.55 - 1.6}{7.15 - 1.2} = 0.495 \text{ g/g}$$

q_s : The specific biomass production rate

$$q_s = \frac{U_{\max}}{Y_{x/s}} = \frac{0.083}{0.495} = 0.167 \text{ g/g.h}$$

$Y_{\%}$: the percent of theriocal biomass yield

$$Y\% = \frac{Y_{x/s}}{0.51} \times 100\% = \frac{0.459}{0.51} \times 100\% = 97\%$$

4. 3 Calculation of kinetics parameter for *E. fibuligera* 5097

U_{mx} : the maximum specific growth rate

$$U_{max} = \frac{\ln(x_2) - \ln(x_1)}{t_2 - t_1} = \frac{1.29 - 0.29}{21 - 9} = 0.082 \text{ h}^{-1}$$

$Y_{x/s}$: the biomass yield

$$Y_{x/s} = \frac{m_x}{m_s} = \frac{4.8 - 2.2}{6.7 - 1.3} = 0.473 \text{ g/g}$$

q_s : The specific biomass production rate

$$q_s = \frac{U_{max-1}}{Y_{x/s}} = \frac{0.082}{0.473} = 0.173 \text{ g/g.h}$$

Protein Y% : the percent of theriocal biomass yield


$$\text{protein Y\%} = \frac{Y_{x/s}}{0.51} \times 100\% = \frac{0.473}{0.51} \times 100\% = 92.7\%$$

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