



APPENDICES

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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APPENDIX A

Preparation of Culture Medium

1. Potato dextrose agar (PDA)

PDA composed of:

| | | |
|----------------------------|-----|-----|
| Potatoes, peeled and diced | 200 | g/l |
| D-Glucose | 20 | g/l |
| Agar | 15 | g/l |

Boil 200 g of peeled, diced potatoes for 1 hr in 1 l of distilled water. Filter, and make up the filtrate to 1 l. Add the glucose and agar and dissolve by steaming. Sterilize by autoclaving at 121°C for 20 min.

2. Yeast malt broth (YMB)

YMB composes of:

| | | |
|---------------|------|-----|
| Yeast extract | 3.0 | g/l |
| Malt extract | 5.0 | g/l |
| Peptone | 5.0 | g/l |
| Glucose | 10.0 | g/l |
| Agar | 20.0 | g/l |

YMB consisted of glucose (100 g/l) was prepared similar to YMB but using 100 g/l of glucose.

APPENDIX B

DNS Method

1. Preparation of DNS solution

DNS solution composes of:

| | | |
|---------------------------|-----|---|
| 3,5-Dinitrosalicylic Acid | 10 | g |
| NaOH | 10 | g |
| Sodium potassium tartrate | 300 | g |

Dissolve 10 g of DNS in 200 ml of NaOH solution (2 mol/l). Heat the solution and stir intensively. Add Sodium potassium tartrate (dissolve 300 g of Sodium potassium tartrate in 500 mL of distilled water). Combine both solutions and stir well. Finally, add distilled water to 1 l.

2. Reducing sugar determination

Reducing sugar can be calculated by equation on chart of standard curve of glucose (Figure B.1), xylose (Figure B.2), mannose, (Figure B.3), and galacturonic acid (Figure B.4).

Calculation of glucose concentration is shown below:

$$\text{Glucose concentration (g/l)} = \frac{A_{540 \text{ nm}}}{0.583} \times \text{dilution}$$

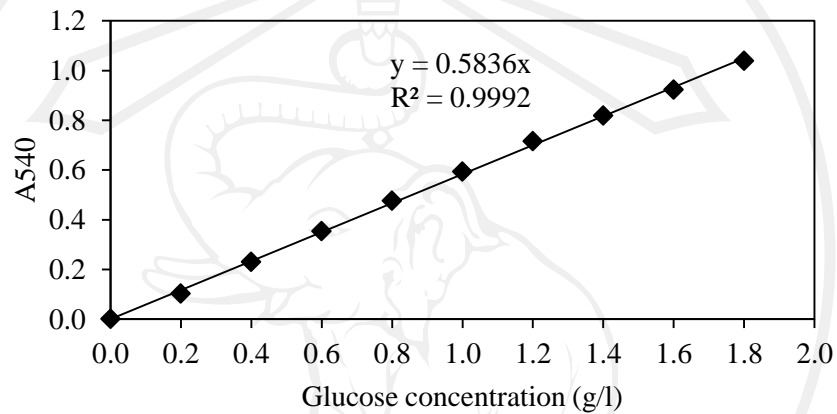


Figure B.1: Glucose standard curve for DNS method.

Calculation of xylose concentration is shown below:

$$\text{Xylose concentration (g/l)} = \frac{A_{540 \text{ nm}}}{0.621} \times \text{dilution}$$

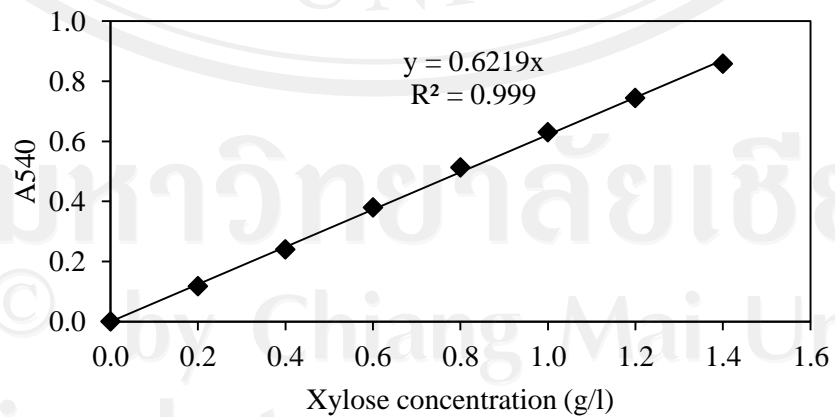


Figure B.2: Xylose standard curve for DNS method.

Calculation of mannose concentration is shown below:

$$\text{Mannose concentration (g/l)} = \frac{A_{540 \text{ nm}}}{0.557} \times \text{dilution}$$

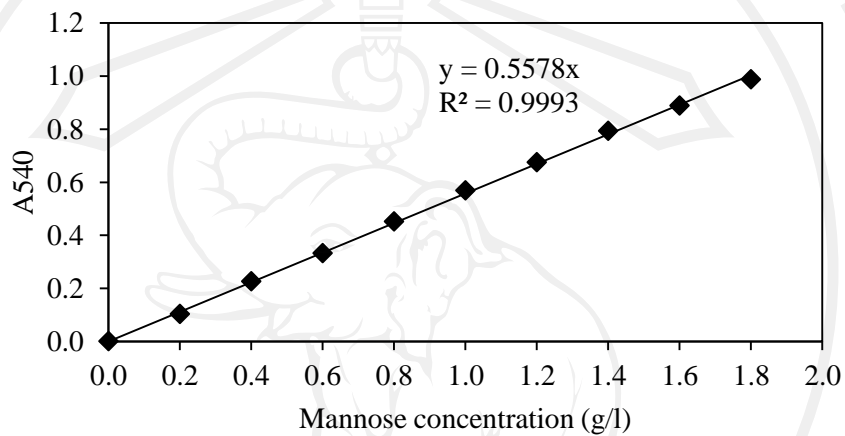


Figure B.3: Mannose standard curve for DNS method.

Calculation of galacturonic acid concentration is shown below:

$$\text{Galacturonic acid concentration (g/l)} = \frac{A_{540 \text{ nm}}}{0.430} \times \text{dilution}$$

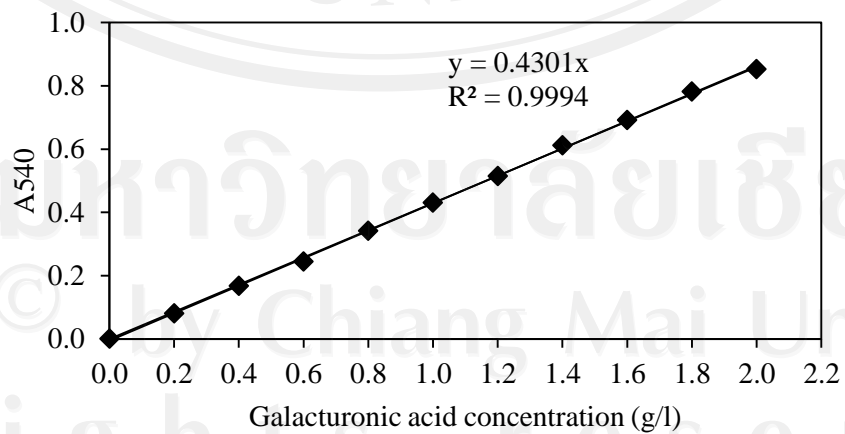


Figure B.4: Galacturonic acid standard curve for DNS method.

APPENDIX C

Total Starch Determination

1. Standard curve of total starch

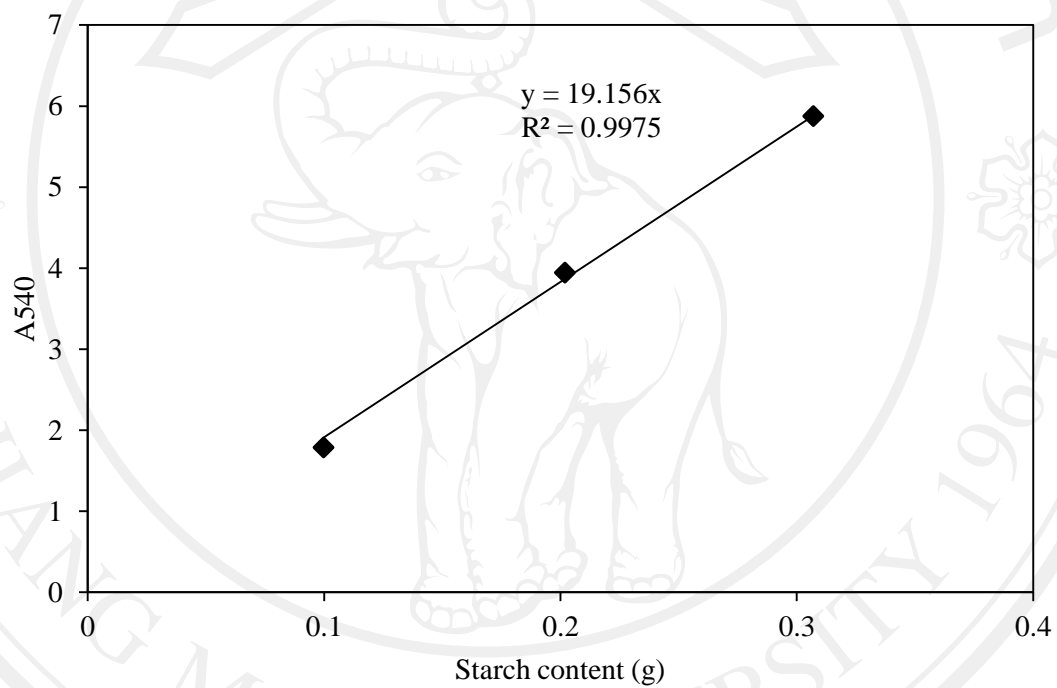


Figure C.1: Starch standard curve for DNS method.

2. Calculation of total starch content

Sample 1.0053 g

After incubated with enzyme

A540 of sample = 0.396, Dilution rate = 50

$$\text{Total A540} = 0.396 \times 50 = 19.8$$

A540 of sample blank = 0.187, Dilution rate = 5

$$\text{Total A540} = 0.187 \times 5 = 0.935$$

A540 of enzyme blank = 0.734, Dilution rate = 20

$$\text{Total A540} = 0.734 \times 20 = 14.68$$

Therefore, Net A540 = 19.8 – 0.935 – 14.68 = 4.185

The equation from starch standard curve (Figure C.1)

$$\text{Starch content (g)} = \frac{\text{A540 nm}}{19.15}$$

$$\text{Starch content (g)} = \frac{4.185}{19.15} = 0.22 \text{ g}$$

Therefore, 1.0053 g of sugar depleted dried longan contained 0.22 g of starch

$$\text{Total starch (\%)} = \frac{0.22}{1.0053} \times 100 = 21.88 \text{ \% (w/w)}$$

APPENDIX D

Results of Reducing sugar concentration

Table D.1: Reducing sugar concentration from fungal treatment.

| Days | Reducing sugar concentration (g/l) | | | | | | | | | |
|-----------|-------------------------------------|------|-------------------------------------|------|--|------|--------------------------------------|------|--------------------------------------|------|
| | <i>Aspergillus niger</i> TISTR 3063 | | <i>Aspergillus niger</i> TISTR 3089 | | <i>Aspergillus foetidus</i> TISTR 3461 | | <i>Trichoderma reesei</i> TISTR 3080 | | <i>Trichoderma reesei</i> TISTR 3081 | |
| | | SD | | SD | | SD | | SD | | SD |
| 0 | 3.31 | 0.28 | 2.21 | 0.14 | 2.07 | 0.17 | 2.96 | 0.10 | 3.06 | 0.04 |
| 1 | 3.86 | 0.44 | 3.37 | 0.07 | 15.50 | 0.94 | 4.00 | 0.43 | 3.24 | 0.28 |
| 2 | 4.88 | 0.13 | 9.08 | 0.13 | 21.60 | 1.18 | 3.74 | 0.15 | 3.46 | 0.10 |
| 3 | 4.98 | 0.17 | 13.72 | 1.01 | 22.00 | 0.93 | 7.52 | 0.17 | 8.14 | 0.70 |
| 4 | 7.23 | 0.20 | 21.72 | 0.04 | 25.55 | 1.49 | 12.66 | 1.00 | 12.08 | 0.24 |
| 5 | 9.50 | 0.05 | 24.03 | 1.77 | 27.07 | 2.57 | 13.58 | 0.67 | 13.58 | 0.67 |
| 6 | 11.34 | 1.17 | 25.10 | 0.55 | 29.80 | 1.69 | 13.71 | 0.85 | 13.86 | 0.47 |
| 7 | 13.94 | 1.63 | 24.96 | 1.03 | 30.84 | 1.02 | 13.19 | 0.48 | 13.99 | 0.72 |
| 8 | 14.93 | 1.17 | 24.47 | 0.31 | 28.64 | 0.66 | 11.82 | 0.23 | 13.08 | 0.45 |
| 9 | 14.49 | 0.54 | 23.25 | 0.78 | 28.71 | 1.59 | 11.03 | 0.29 | 12.49 | 0.32 |
| 10 | 15.00 | 2.05 | 22.29 | 0.77 | 30.03 | 0.62 | 9.02 | 0.45 | 11.57 | 0.54 |

APPENDIX E

Enzyme Activity

1. 0.05 M acetate buffer, pH 5.0 preparation

Combine 14.8 ml of 0.2 M acetic acid which prepare by mixture of 11.55 ml of acetic acid and distilled water 1 l and 35.2 ml of 0.2 M sodium acetate ($C_2H_3O_2Na \cdot 3H_2O$) which prepare by mixture of 27.2 g in distilled water 1 l, dilute the combined to 100 ml and adjust to 500 ml for dilute 0.2 M acetate buffer to 0.05 M acetate buffer and confirm that the pH = 5.0 ± 0.1 (if the pH needs to be adjusted, use acetic acid or sodium acetate solution). It is stored at room temperature. The shelf life of this solution is greater than one year.

2. Calculation

One unit (U) of enzyme activity was defined as the amount of enzyme producing 1 μmol of product per minute under the assay conditions. Therefore, the formula of enzyme activity (U/ml) is described as below:

$$\text{Enzyme activity (U/ml)} = \frac{(a-b-c) \times \text{dilution}}{\text{reaction time (min)} \times \text{volume of sample (ml)}}$$

a = amount of sugar ($\mu\text{mol/ml}$) that release from reaction of sample + substrate

b = amount of sugar ($\mu\text{mol/ml}$) that release from reaction of substrate + buffer

c = amount of sugar ($\mu\text{mol/ml}$) that release from reaction of sample + buffer

Enzymatic activities in this study were determined in U/g dry substrate (U/gds). Therefore, they were converted by the equation below:

$$\text{Enzyme activity (U/gds)} = \frac{\text{volume of total sample (ml)} \times \text{enzyme activity (U/ml)}}{\text{dry substrate (g)}}$$

3. Example of calculation

Calculate amylase activity of *A. foetidus* TISTR 3461 at 3 days cultivation.

a = 2.60 μmol , b = 0.00 μmol , c = 1.94 μmol

dilution = 15, reaction time = 10 min

$$\text{Amylase activity (U/ml)} = \frac{(2.60 - 0.00 - 1.94) \times 15}{10 \text{ min} \times 0.25 \text{ ml}}$$

$$\text{Amylase activity} = 3.96 \text{ U/ml}$$

Sugar deplete dried longan has moisture content about 70% and fifty g of sugar deplete dried longan was used as substrate.

$$\text{Therefore, substrate had dried solids about} = \frac{(100 - 70)}{100} \times 50 = 15 \text{ g}$$

Volume of total sample extract = 50 ml, amylase activity = 6.48 U/ml

$$\text{Amylase activity (U/gds)} = \frac{50 \text{ (ml)} \times 3.96 \text{ (U/ml)}}{15 \text{ (g)}}$$

$$\text{Amylase activity} = 13.2 \text{ U/gds}$$

APPENDIX F

Results of Enzyme Activity

Table F.1: Cellulase activities from fungal cultures.

| Days | Cellulase activity (U/gds) | | | | | | | | | |
|-----------|-------------------------------------|------|-------------------------------------|------|--|------|--------------------------------------|------|--------------------------------------|------|
| | <i>Aspergillus niger</i> TISTR 3063 | | <i>Aspergillus niger</i> TISTR 3089 | | <i>Aspergillus foetidus</i> TISTR 3461 | | <i>Trichoderma reesei</i> TISTR 3080 | | <i>Trichoderma reesei</i> TISTR 3081 | |
| | | SD | | SD | | SD | | SD | | SD |
| 0 | 0.11 | 0.08 | 0.06 | 0.01 | 0.00 | 0.00 | 0.13 | 0.07 | 0.17 | 0.03 |
| 1 | 0.06 | 0.25 | 0.17 | 0.00 | 0.00 | 0.00 | 0.55 | 0.18 | 0.36 | 0.04 |
| 2 | 1.33 | 0.06 | 0.48 | 0.17 | 0.00 | 0.00 | 0.18 | 0.10 | 0.46 | 0.15 |
| 3 | 1.25 | 0.21 | 0.50 | 0.04 | 0.00 | 0.00 | 0.31 | 0.33 | 0.57 | 0.49 |
| 4 | 1.22 | 0.16 | 0.69 | 0.17 | 0.00 | 0.00 | 0.31 | 0.07 | 0.46 | 0.33 |
| 5 | 1.27 | 0.40 | 1.02 | 0.36 | 0.00 | 0.00 | 0.25 | 0.18 | 0.48 | 0.29 |
| 6 | 1.20 | 0.49 | 1.43 | 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.33 | 0.11 |
| 7 | 1.82 | 0.33 | 0.60 | 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.48 | 0.02 |
| 8 | 1.93 | 0.56 | 0.80 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.38 | 0.17 |
| 9 | 1.42 | 0.57 | 0.83 | 0.26 | 0.00 | 0.00 | 0.00 | 0.00 | 0.37 | 0.26 |
| 10 | 1.18 | 0.35 | 0.89 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.22 | 0.15 |

Table F.2: Xylanase activities from fungal cultures.

| Days | Xylanase activity (U/gds) | | | | | | | | | |
|-----------|-------------------------------------|------|-------------------------------------|------|--|------|--------------------------------------|------|--------------------------------------|------|
| | <i>Aspergillus niger</i> TISTR 3063 | | <i>Aspergillus niger</i> TISTR 3089 | | <i>Aspergillus foetidus</i> TISTR 3461 | | <i>Trichoderma reesei</i> TISTR 3080 | | <i>Trichoderma reesei</i> TISTR 3081 | |
| | | SD | | SD | | SD | | SD | | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.16 | 0.15 | 0.14 | 0.16 | 0.00 | 0.00 |
| 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.34 | 1.59 | 0.24 | 0.18 |
| 2 | 3.72 | 0.23 | 5.98 | 0.11 | 2.52 | 0.20 | 1.15 | 0.48 | 0.57 | 0.36 |
| 3 | 3.26 | 0.22 | 3.50 | 0.23 | 3.97 | 0.48 | 1.89 | 0.34 | 1.20 | 0.30 |
| 4 | 3.07 | 0.19 | 3.18 | 0.60 | 3.98 | 0.59 | 2.02 | 0.48 | 1.52 | 0.10 |
| 5 | 3.01 | 0.25 | 3.22 | 0.00 | 5.25 | 1.16 | 2.86 | 0.63 | 2.01 | 0.08 |
| 6 | 2.85 | 0.06 | 3.54 | 0.59 | 3.66 | 0.33 | 1.74 | 0.51 | 1.77 | 0.77 |
| 7 | 3.00 | 0.26 | 3.62 | 0.49 | 3.34 | 0.10 | 1.19 | 0.30 | 1.58 | 0.55 |
| 8 | 2.39 | 0.51 | 3.74 | 0.61 | 3.44 | 1.41 | 1.11 | 0.15 | 1.80 | 0.30 |
| 9 | 2.14 | 0.84 | 3.50 | 0.39 | 1.21 | 0.06 | 1.07 | 0.36 | 1.14 | 0.16 |
| 10 | 1.49 | 0.34 | 1.65 | 0.36 | 1.00 | 0.17 | 0.38 | 0.06 | 0.73 | 0.40 |

Table F.3: Mannanase activities from fungal cultures.

| Days | Mannanase activity (U/gds) | | | | | | | | | |
|-----------|-------------------------------------|------|-------------------------------------|------|--|------|--------------------------------------|------|--------------------------------------|------|
| | <i>Aspergillus niger</i> TISTR 3063 | | <i>Aspergillus niger</i> TISTR 3089 | | <i>Aspergillus foetidus</i> TISTR 3461 | | <i>Trichoderma reesei</i> TISTR 3080 | | <i>Trichoderma reesei</i> TISTR 3081 | |
| | | SD | | SD | | SD | | SD | | SD |
| 0 | 0.17 | 0.03 | 0.30 | 0.04 | 0.00 | 0.00 | 0.34 | 0.17 | 0.42 | 0.18 |
| 1 | 0.97 | 0.16 | 0.13 | 0.07 | 0.00 | 0.00 | 0.61 | 0.37 | 0.52 | 0.05 |
| 2 | 0.88 | 0.03 | 0.34 | 0.05 | 0.00 | 0.00 | 0.58 | 0.04 | 0.54 | 0.13 |
| 3 | 1.22 | 0.09 | 0.64 | 0.28 | 0.00 | 0.00 | 0.73 | 0.11 | 1.65 | 0.27 |
| 4 | 0.88 | 0.15 | 0.65 | 0.42 | 0.00 | 0.00 | 2.26 | 0.55 | 2.20 | 0.50 |
| 5 | 1.07 | 0.19 | 2.38 | 0.04 | 0.22 | 0.14 | 1.89 | 1.11 | 1.91 | 0.28 |
| 6 | 0.99 | 0.07 | 2.51 | 0.79 | 0.47 | 0.09 | 1.65 | 0.02 | 1.44 | 0.08 |
| 7 | 0.97 | 0.09 | 2.76 | 0.05 | 0.60 | 0.09 | 1.08 | 0.12 | 1.33 | 0.19 |
| 8 | 1.37 | 0.38 | 3.80 | 0.72 | 1.51 | 0.19 | 1.19 | 0.57 | 1.32 | 0.28 |
| 9 | 1.25 | 0.28 | 1.02 | 0.80 | 0.91 | 0.57 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Table F.4: Pectinase activities from fungal cultures.

| Days | Pectinase activity (U/gds) | | | | | | | | | |
|-----------|-------------------------------------|------|-------------------------------------|------|--|------|--------------------------------------|------|--------------------------------------|------|
| | <i>Aspergillus niger</i> TISTR 3063 | | <i>Aspergillus niger</i> TISTR 3089 | | <i>Aspergillus foetidus</i> TISTR 3461 | | <i>Trichoderma reesei</i> TISTR 3080 | | <i>Trichoderma reesei</i> TISTR 3081 | |
| | | SD | | SD | | SD | | SD | | SD |
| 0 | 1.48 | 0.09 | 1.15 | 0.04 | 1.17 | 0.35 | 1.64 | 0.09 | 1.63 | 0.33 |
| 1 | 0.95 | 0.17 | 1.03 | 0.17 | 2.33 | 0.69 | 1.38 | 0.35 | 0.63 | 0.03 |
| 2 | 3.41 | 0.24 | 4.11 | 0.48 | 3.73 | 0.79 | 1.18 | 0.72 | 0.99 | 0.36 |
| 3 | 4.00 | 0.34 | 5.53 | 1.22 | 7.04 | 0.64 | 3.34 | 0.71 | 3.47 | 0.39 |
| 4 | 3.24 | 0.41 | 7.25 | 0.97 | 7.54 | 0.66 | 3.63 | 0.92 | 3.87 | 0.70 |
| 5 | 3.83 | 1.07 | 7.40 | 0.53 | 8.79 | 1.10 | 4.88 | 0.38 | 5.22 | 0.12 |
| 6 | 3.60 | 0.82 | 7.42 | 0.95 | 8.56 | 4.02 | 2.97 | 0.32 | 3.74 | 0.32 |
| 7 | 3.73 | 1.20 | 6.32 | 0.72 | 9.26 | 0.06 | 2.71 | 0.33 | 2.90 | 0.91 |
| 8 | 4.05 | 0.47 | 8.05 | 1.03 | 8.51 | 2.90 | 2.77 | 0.72 | 2.94 | 0.55 |
| 9 | 6.50 | 0.58 | 7.94 | 0.30 | 14.59 | 0.82 | 2.43 | 0.27 | 3.06 | 0.57 |
| 10 | 2.99 | 1.12 | 4.78 | 0.70 | 6.43 | 1.41 | 2.39 | 0.52 | 2.49 | 1.06 |

Table F.5: Amylase activities from fungal cultures.

| Days | Amylase activity (U/gds) | | | | | | | | | |
|-----------|-------------------------------------|------|-------------------------------------|------|--|------|--------------------------------------|------|--------------------------------------|------|
| | <i>Aspergillus niger</i> TISTR 3063 | | <i>Aspergillus niger</i> TISTR 3089 | | <i>Aspergillus foetidus</i> TISTR 3461 | | <i>Trichoderma reesei</i> TISTR 3080 | | <i>Trichoderma reesei</i> TISTR 3081 | |
| | | SD | | SD | | SD | | SD | | SD |
| 0 | 0.67 | 0.10 | 0.37 | 0.08 | 0.36 | 0.07 | 0.52 | 0.14 | 0.83 | 0.16 |
| 1 | 0.51 | 0.40 | 2.01 | 1.21 | 7.22 | 2.54 | 0.63 | 0.15 | 0.49 | 0.30 |
| 2 | 1.04 | 0.21 | 2.23 | 0.97 | 21.49 | 1.14 | 0.34 | 0.13 | 0.65 | 0.04 |
| 3 | 0.98 | 0.10 | 3.04 | 0.49 | 13.28 | 1.78 | 1.98 | 0.09 | 1.83 | 0.37 |
| 4 | 0.92 | 0.09 | 4.12 | 0.77 | 8.76 | 0.10 | 2.39 | 0.81 | 1.72 | 0.41 |
| 5 | 1.36 | 0.19 | 4.35 | 0.88 | 8.65 | 1.35 | 3.15 | 0.25 | 3.04 | 0.69 |
| 6 | 1.48 | 0.15 | 6.07 | 1.89 | 7.47 | 2.17 | 2.03 | 0.31 | 2.23 | 0.14 |
| 7 | 1.50 | 0.13 | 4.14 | 1.66 | 6.98 | 1.35 | 1.73 | 0.36 | 1.95 | 1.05 |
| 8 | 2.04 | 1.10 | 4.20 | 1.17 | 7.29 | 2.56 | 1.81 | 0.08 | 3.43 | 0.65 |
| 9 | 1.88 | 0.45 | 4.12 | 0.65 | 4.92 | 0.69 | 1.73 | 0.43 | 2.79 | 0.52 |
| 10 | 0.56 | 0.23 | 3.52 | 0.51 | 4.89 | 1.03 | 1.56 | 0.43 | 2.09 | 0.48 |

APPENDIX G

Dried Cell Weight

1. Standard curve of dried cell weight

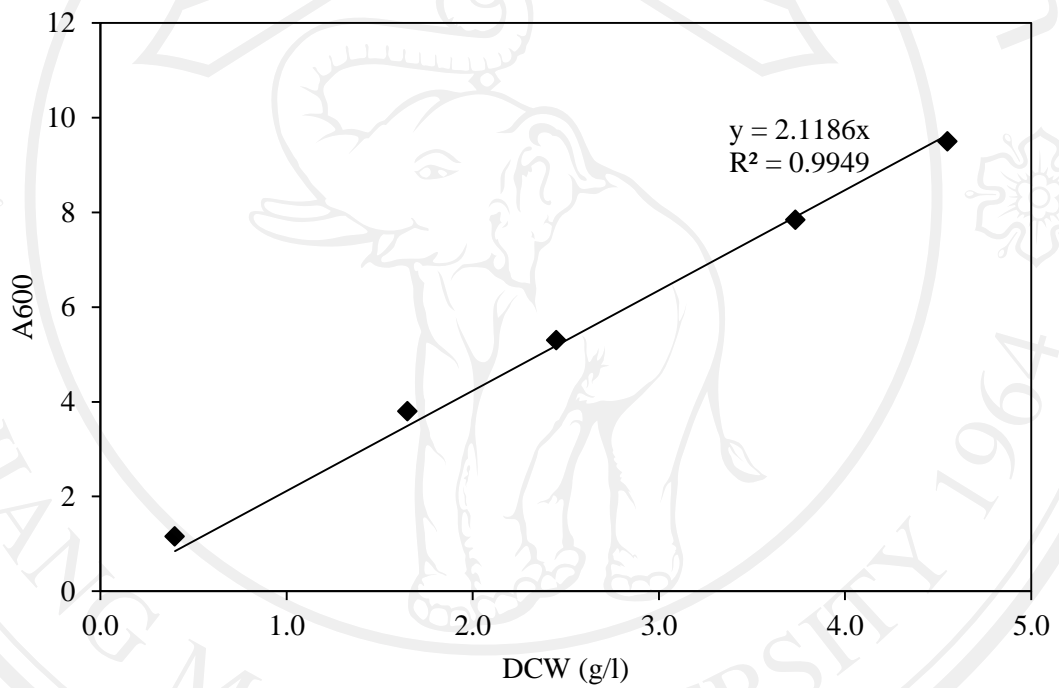


Figure G.1: Standard curve of dried cell weight.

2. Calculation of dried cell weight

Calculation of dried cell weight concentration is shown below:

$$\text{Dried cell weight (g/l)} = \frac{\text{A600 nm}}{2.118} \times \text{dilution}$$

APPENDIX H

HPLC analysis

1. Preparation of mobile phase (5 mM sulfuric acid)

Five mM of sulfuric acid is calculated as follow:

Sulfuric concentration = A %, density = B g/ml

Therefore, 1 ml of sulfuric solution has sulfuric = $\frac{A \times B}{100}$ g

5 mM = 0.005 mol/l, MW of sulfuric acid = 98.08

From equation $\frac{g}{MW} = \text{mol}$

$$g = 98.08 \times 0.005$$

Therefore, 1 l of 5 mM sulfuric acid contained sulfuric acid about =

0.4904 g

$\frac{A \times B}{100}$ g from sulfuric acid A% about 1 ml

0.4904 g from sulfuric acid A% = $\frac{100}{A \times B} \times 0.4904$

Therefore, the volume of sulfuric acid which prepared 1 l of 5 mM sulfuric acid is $\frac{49.04}{A \times B}$

Calculated volume of sulfuric acid and added in to 1 l volumetric flask. After that, DI water is added to adjust solution to 1 l. pH of solution measured pH and filtered by nylon filter membrane with pore size 0.45 μm . Finally, it is degassed by using a trapped vacuum pump.

2. Calculation

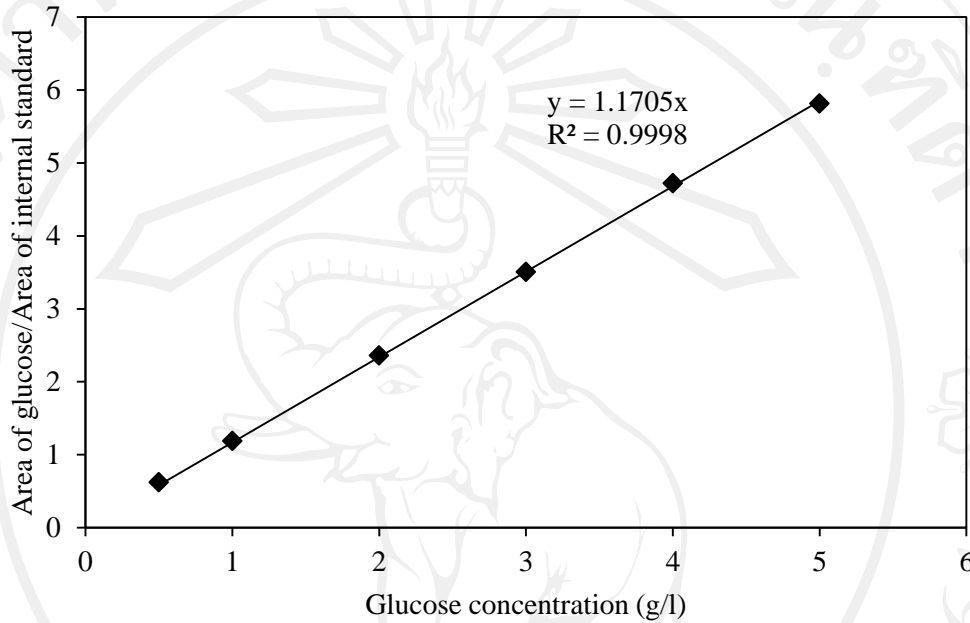


Figure H.1: Glucose standard curve for HPLC analysis.

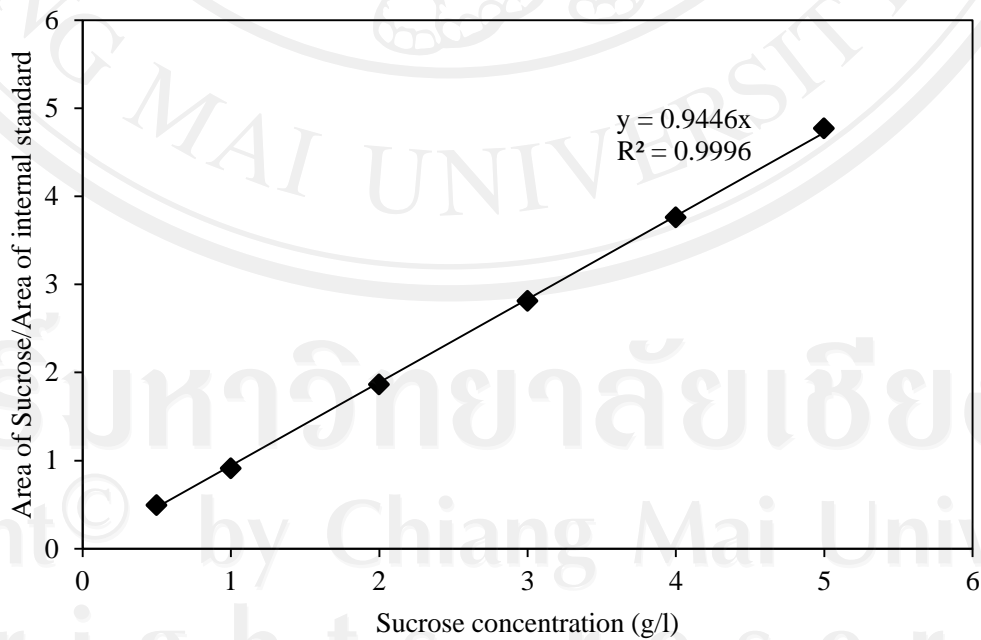


Figure H.2: Sucrose standard curve for HPLC analysis.

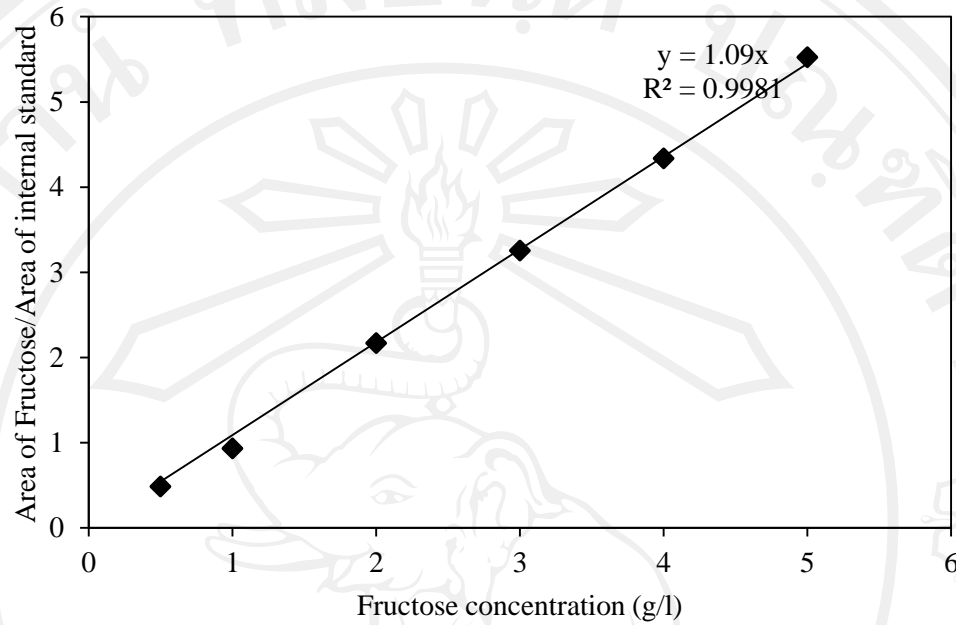


Figure H.3: Fructose standard curve for HPLC analysis.

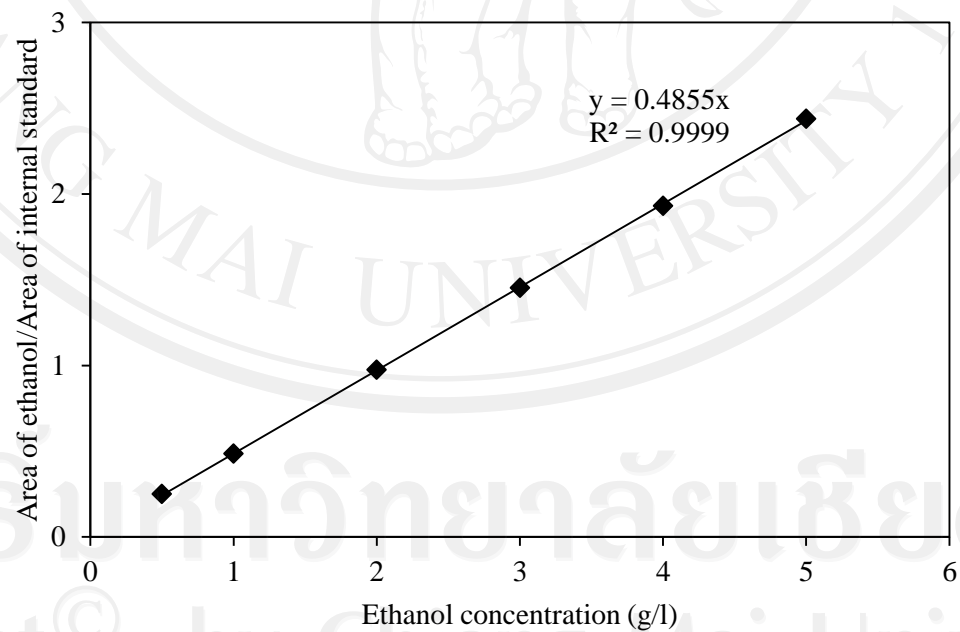


Figure H.4: Ethanol standard curve for HPLC analysis.

Example of calculation

Calculation of glucose concentration from sample of *A. foetidus* TISTR 3461 extract at 0 h-cultivation

Dilution rate = 20

Area of glucose peak = 231178.1

Area of internal standard peak = 69210.1

Therefore area of glucose/area of internal standard

$$= \frac{231178.1}{69210.1} = 3.34$$

Calculated from standard curve (Figure F.1)

$$\text{Glucose concentration (g/l)} = \frac{3.34}{1.170} \times 20$$

Therefore glucose concentration is 57.09 g/l

Calculation of ethanol concentration from sample of *A. foetidus* TISTR 3461 extract at 84 h-cultivation

Dilution rate = 20

Area of ethanol peak = 43786.9

Area of internal standard peak = 78905.6

Therefore, area of ethanol/area of internal standard

$$= \frac{43786.9}{78905.6} = 0.55$$

Calculated from standard curve (Figure F.2)

$$\text{Ethanol concentration (g/l)} = \frac{0.55}{0.485} \times 20$$

Therefore, ethanol concentration is 22.68 g/l

APPENDIX I

Results from Ethanol Production from Fungal Treatment Extract

Table I.1: Dried cell weight.

| Time (h) | Dried cell weight | | | | | | | | | |
|-------------|-------------------|------|------------------|------|---|------|--|------|---|------|
| | YMB | | Glucose solution | | Sugar deplete dried longan extract with glucose | | <i>Aspergillus foetidus</i> TISTR 3461 extract | | <i>Aspergillus niger</i> TISTR 3089 extract | |
| | | SD | | SD | | SD | | SD | | SD |
| 0 | 0.45 | 0.01 | 0.49 | 0.04 | 0.47 | 0.01 | 0.41 | 0.01 | 0.45 | 0.07 |
| 12 | 1.48 | 0.06 | 0.85 | 0.05 | 0.88 | 0.20 | 1.03 | 0.01 | 1.03 | 0.02 |
| 24 | 2.37 | 0.02 | 0.87 | 0.05 | 1.88 | 0.08 | 1.07 | 0.05 | 1.23 | 0.00 |
| 36 | 2.75 | 0.08 | 0.87 | 0.04 | 2.02 | 0.11 | 1.11 | 0.02 | 1.21 | 0.04 |
| 48 | 2.65 | 0.04 | 0.88 | 0.05 | 2.08 | 0.19 | 1.27 | 0.02 | 1.14 | 0.02 |
| 60 | 2.43 | 0.09 | 0.83 | 0.05 | 2.17 | 0.12 | 1.33 | 0.13 | 1.21 | 0.04 |
| 72 | 2.39 | 0.06 | 0.81 | 0.04 | 2.26 | 0.02 | 1.21 | 0.04 | 1.24 | 0.01 |
| 84 | 2.27 | 0.03 | 0.81 | 0.03 | 2.32 | 0.14 | 1.27 | 0.02 | 1.36 | 0.02 |
| 96 | 2.21 | 0.06 | 0.77 | 0.03 | 2.34 | 0.17 | 1.27 | 0.02 | 1.29 | 0.04 |
| 108 | 2.22 | 0.04 | 0.79 | 0.03 | 2.29 | 0.17 | 1.31 | 0.04 | 1.29 | 0.08 |
| 120 | 2.19 | 0.02 | 0.77 | 0.02 | 2.05 | 0.02 | 1.24 | 0.04 | 1.36 | 0.04 |

Table I.2: Fermentable reducing sugar.

| Time (h) | Fermentable reducing sugar | | | | | | | | | |
|-------------|----------------------------|------|------------------|-------|---|------|--|------|---|-------|
| | YMB | | Glucose solution | | Sugar deplete dried longan extract with glucose | | <i>Aspergillus foetidus</i> TISTR 3461 extract | | <i>Aspergillus niger</i> TISTR 3089 extract | |
| | | SD | | SD | | SD | | SD | | SD |
| 0 | 82.15 | 2.54 | 73.90 | 6.88 | 67.85 | 5.06 | 54.36 | 4.87 | 51.68 | 13.43 |
| 12 | 52.19 | 1.14 | 66.72 | 10.05 | 42.75 | 1.90 | 40.99 | 3.02 | 38.34 | 1.19 |
| 24 | 22.74 | 0.93 | 67.33 | 9.30 | 15.31 | 1.53 | 35.90 | 4.89 | 30.39 | 3.56 |
| 36 | 2.23 | 0.36 | 58.32 | 9.31 | 0.04 | 0.12 | 16.08 | 2.97 | 14.18 | 2.62 |
| 48 | 0.00 | 0.11 | 57.61 | 10.28 | 0.00 | 0.12 | 9.60 | 4.73 | 10.42 | 0.94 |
| 60 | 0.17 | 0.05 | 46.32 | 9.59 | 0.28 | 0.27 | 6.18 | 1.15 | 6.67 | 2.62 |
| 72 | 0.08 | 0.11 | 40.11 | 11.05 | 0.03 | 0.17 | 0.00 | 2.43 | 0.00 | 0.19 |
| 84 | 0.27 | 0.04 | 32.46 | 10.64 | 0.11 | 0.07 | 2.62 | 1.52 | 2.87 | 0.87 |
| 96 | 0.20 | 0.09 | 27.79 | 10.21 | 0.46 | 0.10 | 2.09 | 2.82 | 5.79 | 2.00 |
| 108 | 0.25 | 0.11 | 23.10 | 10.78 | 0.28 | 0.09 | 1.68 | 2.87 | 1.63 | 1.62 |
| 120 | 0.31 | 0.10 | 19.17 | 10.54 | 0.22 | 0.19 | 2.47 | 0.15 | 7.77 | 2.81 |

Table I.3: Glucose concentration.

| Time (h) | Glucose concentration | | | | | | | | | |
|-------------|-----------------------|------|------------------|-------|---|------|--|------|---|------|
| | YMB | | Glucose solution | | Sugar deplete dried longan extract with glucose | | <i>Aspergillus foetidus</i> TISTR 3461 extract | | <i>Aspergillus niger</i> TISTR 3089 extract | |
| | | SD | | SD | | SD | | SD | | SD |
| 0 | 81.68 | 1.58 | 78.20 | 3.21 | 73.34 | 1.02 | 57.10 | 0.89 | 50.67 | 0.91 |
| 12 | 60.63 | 1.20 | 69.04 | 10.13 | 46.11 | 2.17 | 37.43 | 0.74 | 33.10 | 0.26 |
| 24 | 23.73 | 1.71 | 62.22 | 15.34 | 15.02 | 1.20 | 27.17 | 1.60 | 23.26 | 1.48 |
| 36 | 2.64 | 0.21 | 53.73 | 3.91 | 0.19 | 0.02 | 12.32 | 1.13 | 11.06 | 0.40 |
| 48 | 0.05 | 0.01 | 49.44 | 4.02 | 0.19 | 0.02 | 3.27 | 0.69 | 3.58 | 1.65 |
| 60 | 0.04 | 0.01 | 46.93 | 14.99 | 0.18 | 0.01 | 1.62 | 0.62 | 0.65 | 0.42 |
| 72 | 0.05 | 0.00 | 40.34 | 10.69 | 0.15 | 0.02 | 1.01 | 0.28 | 0.26 | 0.34 |
| 84 | 0.03 | 0.00 | 35.42 | 8.07 | 0.11 | 0.03 | 0.91 | 0.38 | 0.33 | 0.16 |
| 96 | 0.03 | 0.01 | 31.97 | 10.62 | 0.03 | 0.00 | 0.11 | 0.11 | 0.41 | 0.34 |
| 108 | 0.02 | 0.01 | 24.94 | 11.33 | 0.01 | 0.01 | 0.93 | 0.46 | 0.12 | 0.17 |
| 120 | 0.01 | 0.01 | 20.06 | 10.56 | 0.02 | 0.02 | 1.26 | 0.32 | 0.39 | 0.04 |

Table I.4: Ethanol concentration.

| Time (h) | Ethanol concentration | | | | | | | | | |
|-------------|-----------------------|------|------------------|------|---|------|--|------|---|------|
| | YMB | | Glucose solution | | Sugar deplete dried longan extract with glucose | | <i>Aspergillus foetidus</i> TISTR 3461 extract | | <i>Aspergillus niger</i> TISTR 3089 extract | |
| | | SD | | SD | | SD | | SD | | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12 | 9.88 | 0.18 | 2.50 | 0.18 | 12.08 | 0.62 | 7.56 | 0.20 | 7.12 | 0.23 |
| 24 | 23.67 | 0.77 | 3.86 | 0.29 | 22.86 | 0.92 | 12.28 | 0.93 | 10.73 | 0.07 |
| 36 | 31.57 | 0.25 | 6.53 | 1.65 | 22.07 | 0.21 | 15.86 | 0.20 | 15.22 | 0.35 |
| 48 | 32.06 | 0.22 | 9.28 | 2.14 | 22.43 | 0.40 | 19.18 | 0.32 | 18.57 | 3.40 |
| 60 | 30.49 | 1.15 | 13.13 | 1.36 | 22.36 | 0.60 | 22.51 | 1.19 | 20.19 | 0.43 |
| 72 | 31.68 | 0.08 | 16.98 | 1.64 | 22.05 | 0.95 | 22.59 | 0.64 | 19.20 | 0.06 |
| 84 | 30.84 | 0.23 | 20.36 | 2.09 | 23.80 | 0.15 | 22.88 | 0.34 | 20.07 | 1.11 |
| 96 | 30.39 | 0.67 | 23.36 | 1.09 | 24.38 | 0.49 | 21.17 | 0.34 | 17.93 | 0.37 |
| 108 | 29.57 | 0.30 | 23.68 | 1.19 | 23.92 | 0.58 | 20.28 | 0.30 | 17.32 | 1.09 |
| 120 | 29.40 | 0.31 | 24.92 | 1.16 | 23.52 | 0.49 | 19.43 | 0.18 | 17.53 | 1.08 |

Table I.5: Glucose concentration from the results of sterilized extract and non-sterilized extract.

| Time (h) | Glucose concentration | | | | | | | |
|-------------|---|------|----------------|------|--|------|----------------|------|
| | <i>Aspergillus foetidus</i> TISTR 3461 extract | | | | <i>Aspergillus niger</i> TISTR 3089 extract | | | |
| | Sterilized | | Non-sterilized | | Sterilized | | Non-sterilized | |
| | | SD | | SD | | SD | | SD |
| 0 | 57.10 | 0.89 | 56.52 | 1.52 | 50.67 | 0.91 | 51.61 | 1.88 |
| 12 | 37.43 | 0.74 | 36.10 | 0.74 | 33.10 | 0.26 | 32.81 | 0.91 |
| 24 | 27.17 | 1.60 | 24.19 | 1.20 | 23.26 | 1.48 | 17.91 | 0.58 |
| 36 | 12.32 | 1.13 | 13.09 | 0.74 | 11.06 | 0.40 | 9.39 | 3.83 |
| 48 | 3.27 | 0.69 | 4.40 | 0.34 | 3.58 | 1.65 | 0.50 | 0.05 |
| 60 | 1.62 | 0.62 | 1.47 | 0.28 | 0.65 | 0.42 | 0.10 | 0.14 |
| 72 | 1.01 | 0.28 | 1.22 | 0.83 | 0.26 | 0.34 | 0.13 | 0.02 |
| 84 | 0.91 | 0.38 | 1.44 | 1.15 | 0.33 | 0.16 | 0.75 | 0.18 |
| 96 | 0.11 | 0.11 | 0.70 | 0.61 | 0.41 | 0.34 | 1.10 | 0.20 |
| 108 | 0.93 | 0.46 | 1.19 | 0.77 | 0.12 | 0.17 | 0.13 | 0.12 |
| 120 | 1.26 | 0.32 | 0.76 | 0.52 | 0.39 | 0.04 | 1.32 | 0.09 |

Table I.6: Ethanol concentration from the results of sterilized extract and non-sterilized extract.

| Time (h) | Ethanol concentration | | | | | | | |
|-------------|---|------|----------------|------|--|------|----------------|------|
| | <i>Aspergillus foetidus</i> TISTR 3461 extract | | | | <i>Aspergillus niger</i> TISTR 3089 extract | | | |
| | Sterilized | | Non-sterilized | | Sterilized | | Non-sterilized | |
| | | SD | | SD | | SD | | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.48 | 0.12 |
| 12 | 7.56 | 0.20 | 7.66 | 0.15 | 7.12 | 0.23 | 7.79 | 0.13 |
| 24 | 12.28 | 0.93 | 11.98 | 0.65 | 10.73 | 0.07 | 10.87 | 0.65 |
| 36 | 15.86 | 0.20 | 16.40 | 0.32 | 15.22 | 0.35 | 15.45 | 0.71 |
| 48 | 19.18 | 0.32 | 19.33 | 0.49 | 18.57 | 3.40 | 17.71 | 0.14 |
| 60 | 22.51 | 1.19 | 21.52 | 0.57 | 20.19 | 0.43 | 18.75 | 0.75 |
| 72 | 22.59 | 0.64 | 23.20 | 0.88 | 19.20 | 0.06 | 18.74 | 0.59 |
| 84 | 22.88 | 0.34 | 23.51 | 0.76 | 20.07 | 1.11 | 18.86 | 0.47 |
| 96 | 21.17 | 0.34 | 20.42 | 0.57 | 17.93 | 0.37 | 17.48 | 0.18 |
| 108 | 20.28 | 0.30 | 20.93 | 0.59 | 17.32 | 1.09 | 16.30 | 0.76 |
| 120 | 19.43 | 0.18 | 20.40 | 0.52 | 17.53 | 1.08 | 16.67 | 0.50 |

Table I.7: Glucose concentration from the results of the extract with and without pH-adjusted.

| Time (h) | Glucose concentration | | | | | | | |
|-------------|---|------|---------------------|------|--|------|---------------------|-----|
| | <i>Aspergillus foetidus</i> TISTR 3461 extract | | | | <i>Aspergillus niger</i> TISTR 3089 extract | | | |
| | pH adjusted | | No pH adjustment | | pH adjusted | | No pH adjustment | |
| | | SD | | SD | | SD | | SD |
| 0 | 53.63 | 0.56 | 56.50 | 1.98 | 66.53 | 2.32 | N/A | N/A |
| 12 | 44.15 | 1.50 | 50.45 | 2.78 | 53.61 | 0.81 | N/A | N/A |
| 24 | 26.27 | 0.18 | 32.62 | 0.65 | 31.92 | 0.17 | N/A | N/A |
| 36 | 12.93 | 0.39 | 16.04 | 0.33 | 17.12 | 0.63 | N/A | N/A |
| 48 | 2.40 | 0.07 | 3.40 | 0.03 | 1.59 | 0.32 | N/A | N/A |
| 60 | 0.76 | 0.38 | 2.29 | 0.49 | 1.42 | 0.18 | N/A | N/A |
| 72 | 0.11 | 0.15 | 0.76 | 0.49 | 0.00 | 0.59 | N/A | N/A |
| 84 | 0.38 | 0.57 | 0.77 | 0.38 | 1.54 | 1.05 | N/A | N/A |
| 96 | 0.92 | 0.35 | 0.00 | 0.38 | 1.49 | 1.19 | N/A | N/A |
| 108 | 0.52 | 0.13 | 0.53 | 0.27 | 2.05 | 0.74 | N/A | N/A |
| 120 | 0.00 | 0.38 | 0.31 | 0.20 | 1.29 | 0.22 | N/A | N/A |

Table I.8: Ethanol concentration from the results of the extract with and without pH-adjusted.

| Time (h) | Ethanol concentration | | | | | | | |
|-------------|---|------|---------------------|------|--|------|---------------------|-----|
| | <i>Aspergillus foetidus</i> TISTR 3461 extract | | | | <i>Aspergillus niger</i> TISTR 3089 extract | | | |
| | pH adjusted | | No pH adjustment | | pH adjusted | | No pH adjustment | |
| | | SD | | SD | | SD | | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | N/A | N/A |
| 12 | 6.63 | 0.15 | 3.72 | 0.19 | 8.54 | 0.21 | N/A | N/A |
| 24 | 14.17 | 0.10 | 12.21 | 0.54 | 17.36 | 1.23 | N/A | N/A |
| 36 | 18.78 | 2.33 | 18.47 | 0.79 | 23.20 | 1.48 | N/A | N/A |
| 48 | 24.79 | 0.71 | 24.58 | 0.54 | 26.86 | 1.37 | N/A | N/A |
| 60 | 27.61 | 1.15 | 24.72 | 0.27 | 27.92 | 0.76 | N/A | N/A |
| 72 | 28.04 | 3.38 | 27.80 | 0.86 | 26.72 | 1.16 | N/A | N/A |
| 84 | 26.02 | 2.73 | 27.79 | 0.61 | 26.28 | 2.36 | N/A | N/A |
| 96 | 25.68 | 1.31 | 25.07 | 1.35 | 26.54 | 1.00 | N/A | N/A |
| 108 | 24.83 | 0.14 | 25.14 | 1.61 | 25.24 | 1.60 | N/A | N/A |
| 120 | 25.53 | 0.51 | 26.47 | 0.42 | 25.94 | 0.17 | N/A | N/A |

CURRICULUM VITAE

| | |
|----------------------------|---|
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