# APPENDICES

# APPENDIX A

## Analytical methods

**Appendix A-1** Qualitative analysis of xylooligosaccharides (XOs) by thin layer chromatography (Kubata et al., 1994)

**Conditions**

| | |
|---|---|
| **Stationary phase** | Thin layer chromatography (TLC) (TLC silica gel 60 $F_{254}$, Merck, Germany) |
| **Mobile phase** | 2:1:1 (v/v/v) *n*-butanol : acetic acid : water |

1. Spot a 5.0 µL of standard XOs or XOs sample on TLC plate.
2. Develop the TLC plate with mobile phase as described above.
3. Spray the plate with the mixer of methanol : sulfuric acid ($H_2SO_4$) (1:1 (v/v)).
4. Detect the resolved sugar by heating TLC plate at 100°C for 10 min.

**Appendix A-2** Quantitative analysis of xylanase-hydrolyzed sugars and lactic acid by high performance liquid chromatography (Boonchuay et al., 2014)

**Conditions**

| | |
|---|---|
| **System controller** | SCL-10Avp (Shimadzu, Japan) |
| **Column** | Aminex HPX 87H (300×7.8 mm; Bio-Rad, USA) |
| **Detector** | RI (refractive index detector RID-10A) |
| **Flow rate** | 0.45 mL/min |
| **Oven temperature** | 40°C |
| **Mobile phase** | 5.00 mM sulfuric acid ($H_2SO_4$) |
| **Injection volume** | 20 µL |
| **Total run time** | 25 min |

**Appendix A-3** Quantitative analysis of fermentable sugars and ethanol by high performance liquid chromatography (Aguiar et al., 2005; Qureshi et al., 2015)

**Conditions**

| | |
|---|---|
| **System controller** | SCL-10Avp (Shimadzu, Japan) |
| **Column** | Aminex HPX 87H (300×7.8 mm; Bio-Rad, USA) |
| **Detector** | RI (refractive index detector RID-10A) |
| **Flow rate** | 0.6 mL/min |
| **Oven temperature** | 40°C |
| **Mobile phase** | 5.00 mM sulfuric acid ($H_2SO_4$) |
| **Injection volume** | 20 µL |
| **Total run time** | 20 min |

**Appendix A-4** Xylanase activity assay (Chaiyaso et al., 2011)

**Reagents**

1. Dinitrosalicylic acid (DNS) solution
2. Xylose (Sigma, USA)
3. Beech wood xylan (Sigma, USA)
4. 0.1 M potassium-phosphate buffer (pH 6.5)

**Methods**

**Preparation of calibration curve**

1. Prepare the standard xylose solution in the range of 0–1.0 mg/mL (0.67–6.67 µmol/mL) concentration from 1.0 mg/mL stock solution.
2. Pipette 100 µL of standard xylose solution from each concentration and 300 µL of DNS solution in microtube.
3. Mix well by vortex mixer and boil for 10 min.
4. Stop the reaction immediately by standing the microtube in ice bath for 10 min.
5. Add 600 µL of distilled water in the reaction mixture and mix well by vortex mixer.
6. Measure at absorbance 540 nm.

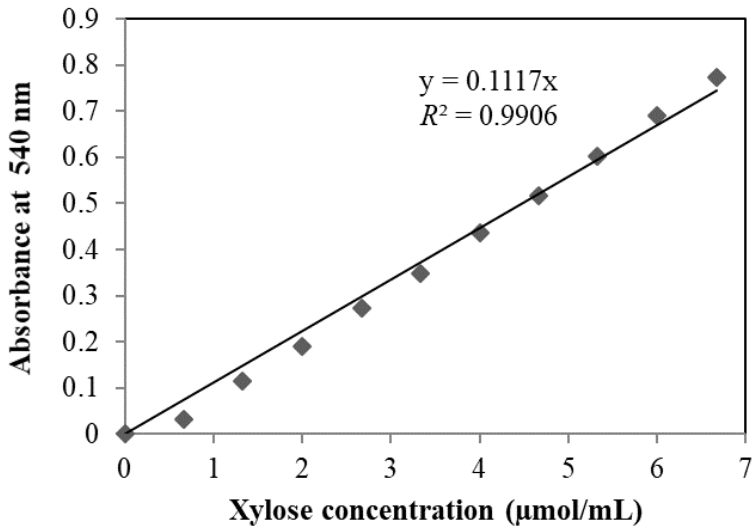


**Figure A-1** Calibration curve of xylose concentration(µmol/mL) and absorbance at 540 nm.

**Xylanase activity assay**

**Substrate:** 1.0% (w/v) beech wood xylan solution in 0.1 M potassium-phosphate buffer (pH 6.5)

**Buffer:** 0.1 M potassium-phosphate buffer (pH 6.5)

**Table A-1** Reaction mixture of xylanase activity assay.

| Reaction mixture | Enzyme (µL) | Substrate (µL) | Buffer (µL) |
|---|---|---|---|
| Control | - | - | 100 |
| Enzyme + Substrate (ES) | 50 | 50 | - |
| Enzyme + Buffer (EB) | 50 | - | 50 |
| Substrate + Buffer (SB) | - | 50 | 50 |

1. Pipette the reaction mixture in microtube (Table A-1) and incubate at 50°C on water bath for 10 min.
2. Stop the reaction by adding 300 µL of DNS solution in microtube.
3. Mix well by vortex mixer and boil for 10 min.
4. Stop the reaction immediately by standing the microtube in ice bath.

5. Add 600 µL of distilled water in the reaction mixture and mix well by vortex mixer.
6. Measure at absorbance 540 nm.

**Calculation**

Net absorbance (Abs) = ES-(EB+SB)

$$\text{Xylanase activity (U/mL)} = \frac{\text{Net Abs}}{\text{Slope} \times 10\ (\text{min}) \times \text{enzyme (mL)}}$$

One unit of xylanase activity (U) is defined as the amount of enzyme liberating 1.0 µmol of reducing sugar (as xylose) per min under assay conditions (Chaiyaso et al., 2011).

**Appendix A-5** Endo-glucanase activity assay (Zhang et al., 2009)

**Reagents**

1. Dinitrosalicylic acid (DNS) solution
2. Glucose (Sigma, USA)
3. Sodium carboxymethyl cellulose (Sigma, USA)
4. 0.1 M Sodium-citrate buffer (pH 5.0)

**Methods**

**Preparation of calibration curve**



1. Prepare the standard glucose solution in the range of 0–1 mg/mL (0.56–5.56 µmol/mL) concentration from 1 mg/mL stock solution.
2. Pipette 100 µL of standard glucose solution from each concentration and 300 µL of DNS solution in microtube.
3. Mix well by vortex mixer and boil for 10 min.
4. Stop the reaction immediately by standing the microtube in ice bath for 10 min.
5. Add 600 µL of distilled water in the reaction mixture and mix well by vortex mixer.
6. Measure at absorbance 540 nm.

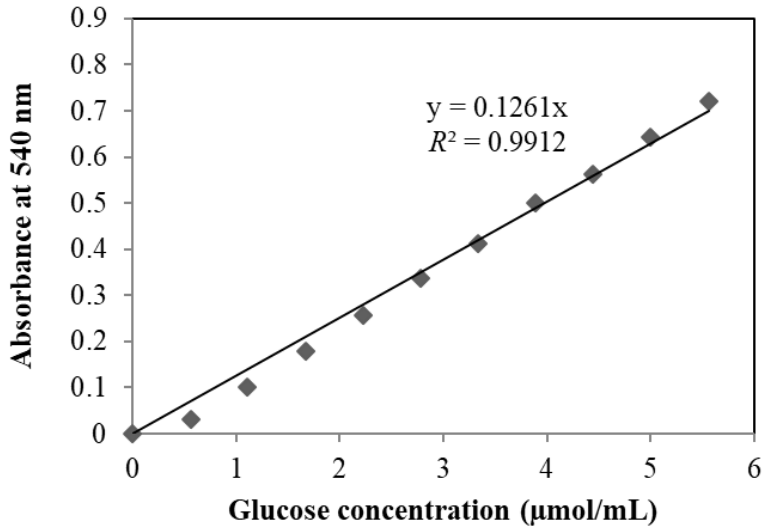


**Figure A-2** Calibration curve of glucose concentration (µmol/mL) and absorbance at 540 nm.

**Endo-glucanase activity assay**

**Substrate:** 0.5% (w/v) sodium carboxymethyl cellulose (CMC) solution in 0.1M sodium-citrate buffer (pH 5.0)

**Buffer:** 0.1 M sodium-citrate buffer (pH 5.0)

**Table A-2** Reaction mixture of endo-glucanase activity assay

| Reaction mixture | Enzyme (µL) | Substrate (µL) | Buffer (µL) |
|---|---|---|---|
| Control | - | - | 100 |
| Enzyme + Substrate (ES) | 50 | 50 | - |
| Enzyme + Buffer (EB) | 50 | - | 50 |
| Substrate + Buffer (SB) | - | 50 | 50 |

1. Pipette the reaction mixture in microtube (Table A-2) and incubate at 50°C on water bath for 10 min.
2. Stop the reaction by adding 300 µL of DNS solution in microtube.
3. Mix well by vortex mixer and boil for 10 min.
4. Stop the reaction immediately by standing the microtube in ice bath.

5. Add 600 µL of distilled water in the reaction mixture and mix well by vortex mixer.
6. Measure at absorbance 540 nm.

**Calculation**

Net absorbance (Abs) = ES-(EB+SB)

$$\text{Endo-glucanase activity (U/mL)} = \frac{\text{Net Abs}}{\text{Slope} \times 10\ (\text{min}) \times \text{enzyme (mL)}}$$

One unit of endo-glucanase activity (U) is defined as the amount of enzyme liberating 1.0 µmol of reducing sugar (as glucose) per min under assay conditions (Zhang et al., 2009).

**Appendix A-6** Total cellulase activity assay (Filter paper assay) (Ghose, 1987)

**Reagents**

1. Dinitrosalicylic acid (DNS) solution
2. Whatman No. 1 filter paper strip (GE Health Care, UK)
3. 0.1 M sodium-citrate buffer (pH 5.0)

**Methods**

**Total cellulase activity assay**

**Substrate:** Whatman No. 1 filter paper strip, 0.1×0.6 cm (≈ 5 mg)

**Buffer:** 0.1 M sodium-citrate buffer (pH 5.0)



**Table A-3** Reaction mixture of total cellulase activity assay

| Reaction mixture | Enzyme (µL) | Substrate (mg) | Buffer (µL) |
|---|---|---|---|
| Control | - | - | 100 |
| Enzyme + Substrate (ES) | 50 | 5 | 50 |
| Enzyme + Buffer (EB) | 50 | - | 50 |
| Substrate + Buffer (SB) | - | 5 | 100 |

1. Pipette the reaction mixture in microtube (Table A-3) and incubate at 50°C on water bath for 60 min.
2. Stop the reaction by adding 300 µL of DNS solution in microtube.
3. Mix well by vortex mixer and boiling for 10 min.
4. Stop the reaction immediately by stand the test tube in ice bath.
5. Add 600 µL of distilled water in the reaction mixture and mix well by vortex mixer.
6. Stand the test tube for 20 min
7. Measure at absorbance 540 nm

**Calculation**

Net absorbance (Abs) = ES-(EB+SB)

$$\text{Endoglucanase activity (U/mL)} = \frac{\text{Net Abs}}{\text{Slope} \times 60\ (\text{min}) \times \text{enzyme (mL)}}$$

One unit of total cellulase activity (U) is defined as the amount of enzyme liberating 1.0 µmol of reducing sugar (as glucose) per min under assay conditions

**Appendix A-7** β-glucosidase activity assay (Lasrado and Gudipati, 2013; Salma, 2008)

**Reagents**

1. *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG) (Sigma, USA)
2. Sodium tetraborate (RCI Labscan, Thailand)

**Substrate:** 5 mM *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG) in 0.1 M sodium-citrate buffer pH 5.0

**Buffer:** 0.1 M sodium-citrate buffer (pH 5.0)

1. Pipette 100 µL of enzyme and 900 µL of *p*NPG in microtube.
2. Mix well by vortex mixer and incubate at 50°C for 10 min.
3. Stop the reaction by the addition of 0.1 mL saturated solution of sodium tetraborate with a final volume of 1.1 mL
4. Measure the releasing of *p*-nitrophenol at absorbance 400 nm.

The concentration of *p*-nitrophenol was calculated by Lambert and Bear equation:

$$A = \frac{\Delta E}{\varepsilon \times d \times C}$$

Where:

| | | |
|---|---|---|
| A | = | Activity (U/mL) |
| △E | = | Absorbance at 400 nm |
| ε | = | Molar extinction coefficient (L/mol/cm) |
| d | = | Cuvette width (cm) |
| C | = | Amount of enzyme (mL) |

The concentration of *p*-nitrophenol released was calculated using the molar extinction coefficient, ε 400 =18,300 L/mol/cm (Salma, 2008).

**Appendix A-8** Protein measurement by Lowry method (Lowry et al., 1951)

**Chemicals**

1. **Reagent I:**
   - 0.1 mL of 5% (w/v) copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) solution
   - 0.9 mL of potassium sodium tartrate solution (1 g in 90 mL of distilled water)
   - 50 mL of 2% (w/v) sodium carbonate ($Na_2CO_3$) in 0.1 M sodium hydroxide (NaOH) solution
2. **Reagent II:**
   - Folin-Ciocalteu reagent (Merck, Germany) and deionized water (1:1, v/v)

**Methods**

**Preparation of calibration curve**

1. Prepare the standard tyrosine solution in the rage of 0–1.0 mg/mL concentration from 1 mg/mL stock solution.

2. Pipette 0.25 mL of standard tyrosine solution (0–1.0 mg/mL concentration) and 2.5 mL of Reagent I (freshly prepare) in tube.
3. Mix well by vortex mixer and incubate for 10 min at room temperature.
4. Add 0.25 mL of Reagent II in the reaction mixture and immediately mix by vortex mixer.
5. Incubate for 30 min at room temperature.
6. Measure at absorbance 600 nm.

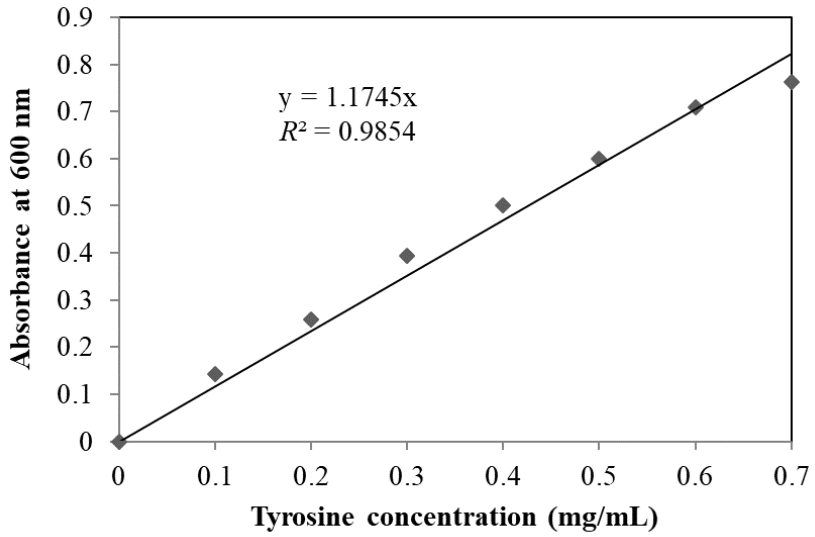


**Figure A-3** Calibration curve of tyrosine concentration (mg/mL) and absorbance at 600 nm.

**Protein measurement**

1. Pipette 0.25 mL of sample and 2.5 mL of Reagent I (freshly prepare) in tube.
2. Mix well by vortex mixer and incubate for 10 min at room temperature.
3. Add 0.25 mL of Reagent II in the reaction mixture and immediately mix by vortex mixer.
4. Incubate for 30 min at room temperature.
5. Measure at absorbance 600 nm.

**Appendix A-9** Kinetic parameters of purified xylanase from *Streptomyces thermovulgaris* TISTR1948.

The fitting of the kinetic model for purified xylanase from *S. thermovulgaris* TISTR1948 was tested according to the method of Leksawasdi (2016).

**Table A-4** The kinetic data obtained from the reaction catalyzed by purified xylanase from *Streptomyces thermovulgaris* TISTR1948 using beech wood xylan as the substrate.

| **[S]** **(mg/mL)** | **$v_0$*** **($U/mg_{protein}$)** | **1/[S]** **(mL/mg)** | **1/v** **($mg_{protein}/U$)** |
|---|---|---|---|
| 0.1 | 0.80 | 10.00 | 1.244 |
| 0.2 | 2.53 | 5.00 | 0.395 |
| 0.3 | 4.03 | 3.33 | 0.248 |
| 0.4 | 5.02 | 2.50 | 0.199 |
| 0.5 | 5.20 | 2.00 | 0.192 |
| 0.6 | 5.90 | 1.67 | 0.170 |
| 0.7 | 6.32 | 1.43 | 0.158 |
| 0.8 | 7.52 | 1.25 | 0.133 |
| 0.9 | 7.63 | 1.11 | 0.131 |
| 1.0 | 8.64 | 1.00 | 0.116 |
| 2.0 | 10.07 | 0.50 | 0.099 |
| 4.0 | 11.26 | 0.25 | 0.089 |
| 8.0 | 12.60 | 0.13 | 0.079 |
| 10.0 | 14.68 | 0.10 | 0.068 |
| 20.0 | 15.33 | 0.05 | 0.065 |

* $v_0$ = $U/mg_{protein}$ or $\mu mol/min/mg_{protein}$

**Table A-5** Calculation of residual sum of squares (RSS) and regression sum of squares (RegSS) for the plot to regress the data from Table A-4.

| **[S] (mg/mL)** | **Actual $v_0$ ($U/mg_{protein}$)** | **Predicted $v_0$ ($U/mg_{protein}$)** | **(Predicted $v_0$)$^2$ $(U/mg_{protein})^2$** | **(Actual $v_0$- Predicted $v_0$)$^2$ $(U/mg_{protein})^2$** |
|---|---|---|---|---|
| 0.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.1 | 0.80 | 1.63 | 2.65 | 0.68 |
| 0.2 | 2.53 | 2.92 | 8.50 | 0.15 |
| 0.3 | 4.03 | 3.96 | 15.66 | 0.01 |
| 0.4 | 5.02 | 4.82 | 23.21 | 0.04 |
| 0.5 | 5.20 | 5.54 | 30.69 | 0.12 |
| 0.6 | 5.90 | 6.16 | 37.89 | 0.07 |
| 0.7 | 6.32 | 6.69 | 44.71 | 0.14 |
| 0.8 | 7.52 | 7.15 | 51.10 | 0.14 |
| 0.9 | 7.63 | 7.55 | 57.07 | 0.01 |
| 1 | 8.64 | 7.91 | 62.64 | 0.53 |
| 2 | 10.07 | 10.07 | 101.46 | 0.00 |
| 4 | 11.26 | 11.66 | 136.03 | 0.16 |
| 8 | 12.60 | 12.66 | 160.35 | 0.00 |
| 10 | 14.68 | 12.88 | 165.99 | 3.24 |
| 20 | 15.33 | 13.34 | 177.96 | 3.96 |
| | | **SUM** | **1075.15** | **9.198** |
| | | | **RegSS** | **RSS** |

**Table A-6** Calculation of residual sum of squares (RSS), regression sum of squares (RegSS), mean squares (MS) and the corresponding correlation coefficient ($R^2$) results after fitting the plot to the experimental data from Table A-5.

| **Data** | **RegSS** | **RSS** | **RegSS + RSS** | **$N_{Residual}$*** | **MS**** | ***$R^2$***** |
|---|---|---|---|---|---|---|
| ν from Table A-5 | 1076.15 | 9.20 | 1085.35 | 13 | 0.7075 | 0.9915 |

*$N_{Residual}$ = 15 - 2 = 13

**MS = $RSS/N_{Residual}$; MS = 9.20/13 = 0.7075

***$R^2$ = RegSS/(RegSS+RSS) = 1075.90 /(1085.35) = 0.9915

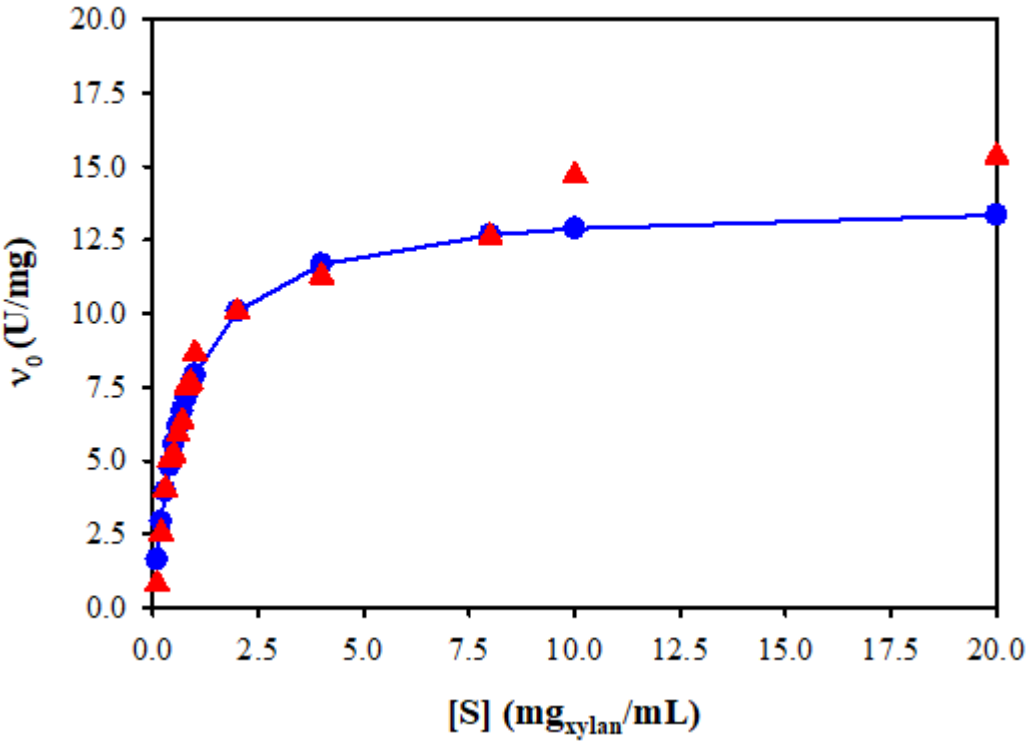


**Figure A-4** Michaelis–Menten plot of actual $v_0$ (▲) by the purified xylanase from *Streptomyces thermovulgaris* TISTR1948 and predicted $v_0$ (●).

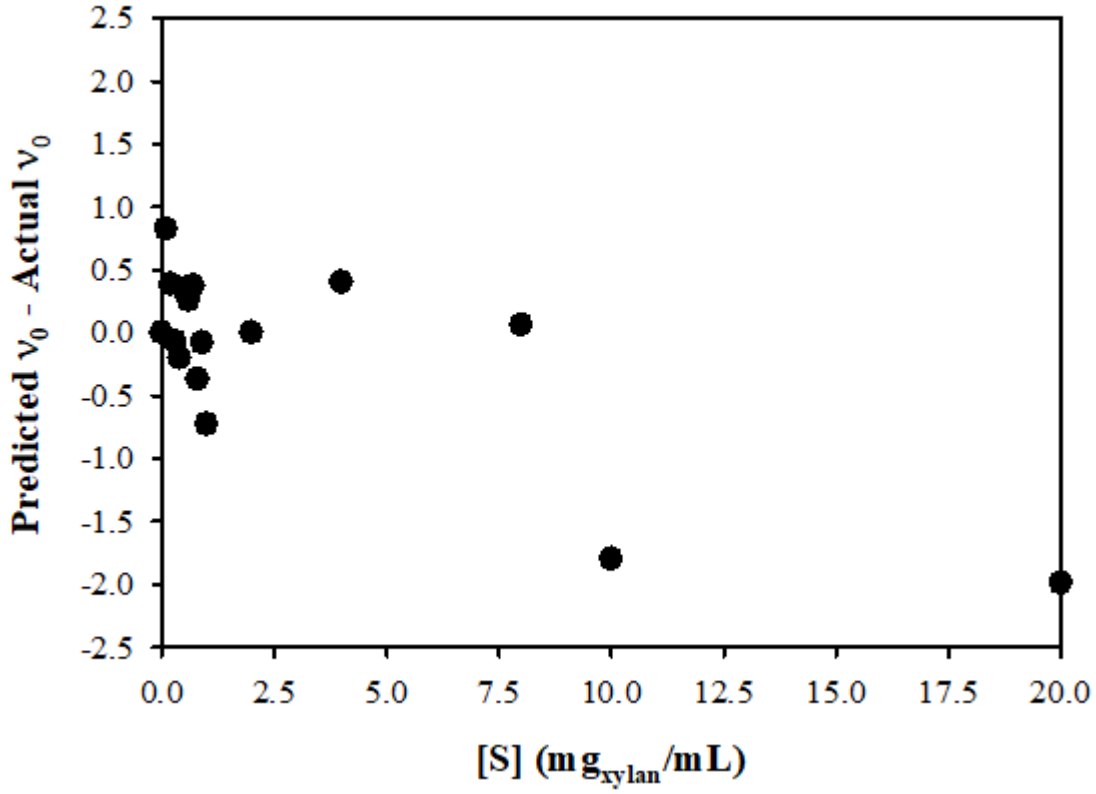


**Figure A-5** Scatter plot of the residual from Figure A-4.

**Table A-7** The $K_m$ and $V_{max}$ results after using goal seek function in Microsoft Excel version 2016.

| **Goal seek turn** | **RSS before using goal seek** | **Parameters** | **Value before using goal seek** | **Value after using goal seek** | **RSS after using goal seek** | **Deducted RSS** |
|---|---|---|---|---|---|---|
| 0 | 9.198 | - | - | - | - | - |
| 1.1 | 9.198 | $K_m$ | 0.750 | 0.741 | 9.184 | 1.288 |
| 1.2 | 9.184 | $V_{max}$ | 13.850 | 14.681 | 7.910 | |
| 2.1 | 7.910 | $K_m$ | 0.741 | 0.860 | 5.741 | 2.493 |
| 2.2 | 5.741 | $V_{max}$ | 14.681 | 15.011 | 5.416 | |
| 3.1 | 5.416 | $K_m$ | 0.860 | 0.912 | 5.301 | 0.223 |
| 3.2 | 5.301 | $V_{max}$ | 15.011 | 15.161 | 5.193 | |
| 4.1 | 5.193 | $K_m$ | 0.912 | 0.914 | 5.193 | 0.000 |
| 4.2 | 5.193 | $V_{max}$ | 15.161 | 15.171 | 5.193 | |

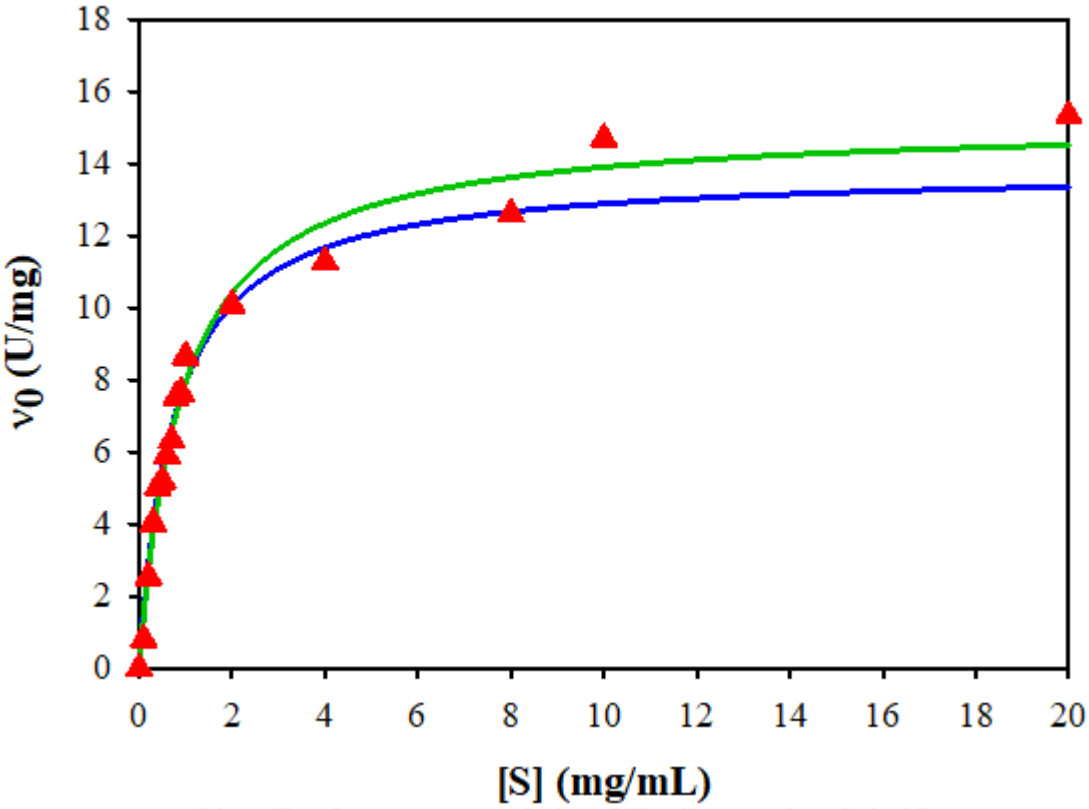


**Figure A-6** Michaelis–Menten plot of actual $v_0$ (▲) by the purified xylanase from *Streptomyces thermovulgaris* TISTR1948, predicted $v_0$ (—) and predicted $v_0$ after using goal seek function (—).

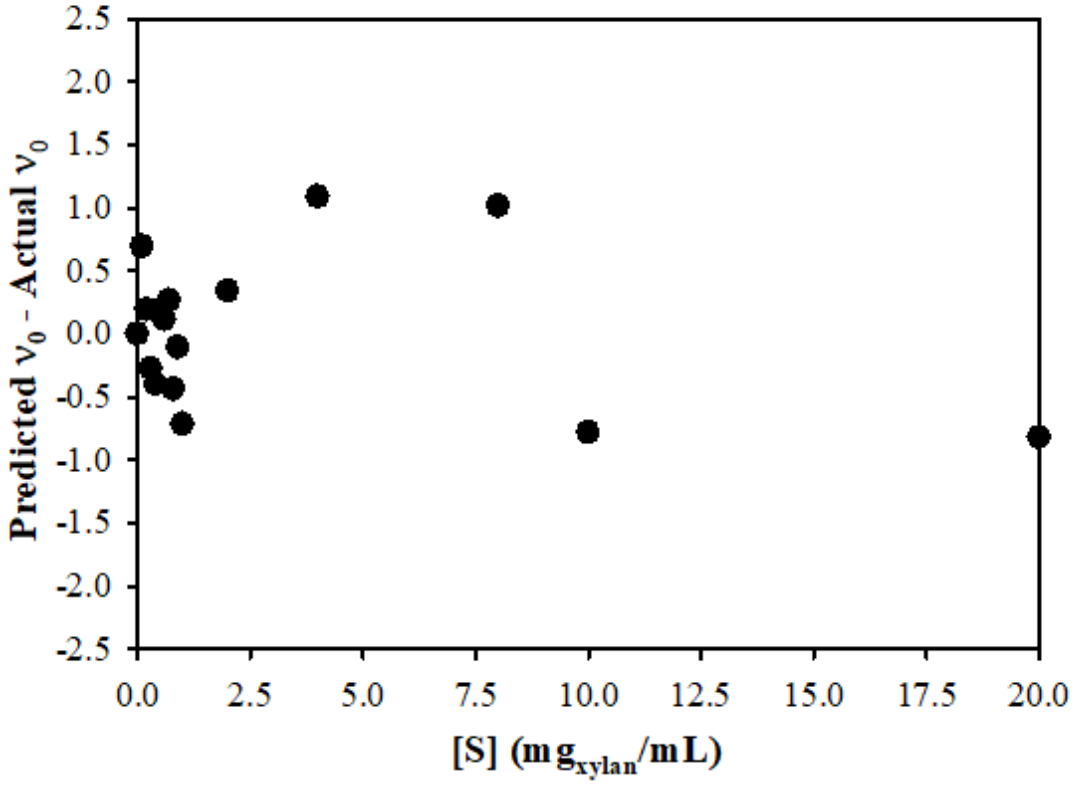


**Figure A-7** Scatter plot of the residual from Figure A-6.

**Table A-8** Calculation of residual sum of squares (RSS) and regression sum of square (RegSS) using the $K_m$ and $V_{max}$ from goal seek function.

| **[S] (mg/mL)** | **Actual $v_0$ ($U/mg_{protein}$)** | **Predicted $v_0$ ($U/mg_{protein}$)** | **(Predicted $v_0$)$^2$ $(U/mg_{protein})^2$** | **(Actual $v_0$- Predicted $v_0$)$^2$ $(U/mg_{protein})^2$** |
|---|---|---|---|---|
| 0.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.1 | 0.80 | 1.49 | 2.23 | 0.48 |
| 0.2 | 2.53 | 2.72 | 7.41 | 0.04 |
| 0.3 | 4.03 | 3.75 | 14.03 | 0.08 |
| 0.4 | 5.02 | 4.61 | 21.30 | 0.16 |
| 0.5 | 5.20 | 5.36 | 28.74 | 0.03 |
| 0.6 | 5.90 | 6.01 | 36.10 | 0.01 |
| 0.7 | 6.32 | 6.58 | 43.24 | 0.07 |
| 0.8 | 7.52 | 7.08 | 50.09 | 0.20 |
| 0.9 | 7.63 | 7.52 | 56.60 | 0.01 |
| 1 | 8.64 | 7.92 | 62.77 | 0.52 |
| 2 | 10.07 | 10.41 | 108.35 | 0.11 |
| 4 | 11.26 | 12.35 | 152.45 | 1.17 |
| 8 | 12.60 | 13.61 | 185.35 | 1.02 |
| 10 | 14.68 | 13.90 | 193.20 | 0.62 |
| 20 | 15.33 | 14.51 | 210.47 | 0.68 |
| | | **SUM** | 1172.31 | 5.193 |
| | | | **RegSS** | **RSS** |



**Table A-9** Calculation of residual sum of squares (RSS), regression sum of squares (RegSS), mean squares (MS) and the corresponding correlation coefficient ($R^2$) result after fitting the plot to the experimental data from Table A-8.

| **Data** | **RegSS** | **RSS** | **RegSS + RSS** | **$N_{Residual}$** | **MS** | **$R^2$** |
|---|---|---|---|---|---|---|
| v from Table A-8 | 1172.31 | 5.19 | 1177.51 | 13 | 0.3994 | 0.9956 |

**Appendix A-10** Acid-insoluble lignin analysis (TAPPI T-222-om-02) (Technical Association of the Pulp and Paper Industry; TAPPI, 2006 ).

**Instruments**

1. Filtration apparatus (Figure A-8), consisting of:
   - Filtering flask (2,000 mL)
   - Filtering crucible
   - Adapter and a siphon tube

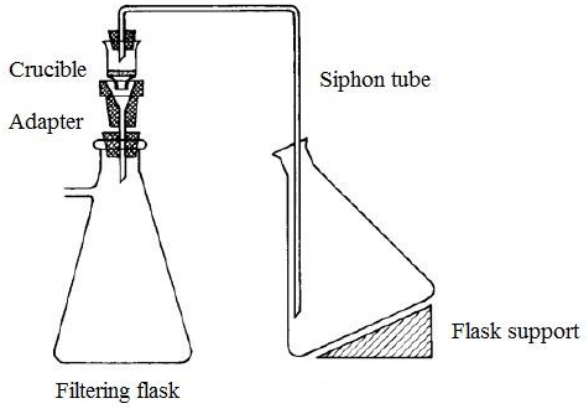


**Figure A-8** Lignin filtration apparatus (TAPPI T-222-om-02).
**Source:** Technical Association of the Pulp and Paper Industry; TAPPI (2006)

2. Filtering crucibles (dry the filtering crucibles in an oven at 105°C for 2 h, cool, and weigh before use)
3. Beaker (100 mL)
4. Erlenmeyer flasks (1.0 L, mark at 575 mL volume)
5. Reflux condenser attached to the flask (optional)
6. Drying oven (forced circulation type, maintain at 105°C)

**Chemicals**

1. 72% Sulfuric acid ($H_2SO_4$) solution
2. Ethanol-benzene mixture (1:2 (v/v))

**Methods**

1. Allow the wood sample to reach moisture equilibrium in the atmosphere, and weigh out two test specimens to 1.0±0.1 g.
2. Place the test specimens in 100 mL beaker and keep the beaker in a bath at 2±1°C during the acid addition.
3. Add 15 mL of cold (10–15°C) 72% sulfuric acid ($H_2SO_4$) to the beakers containing the sample, stir and macerate the sample by using the glass rod until the specimen is dispersed.
4. Cover the beaker with a watch glass, keep at 20±1°C and stir for 2 h.
5. Transfer the reaction mixture from the beaker to flask containing 300–400 mL of water.
6. Rinse the beaker with water and adjust the volume of reaction mixture in flask to 575 mL (the final concentration of sulfuric acid is 3.0%).
7. Boil the reaction mixture for 4 h by using a reflux condenser to maintain a constant volume.
8. Incline the flask to allow the settling of insoluble material (lignin). (If the lignin is still dispersed, place the flask in this position for overnight.)
9. Decant or siphon off the supernatant solution through a filtering crucible without stirring up the precipitate.
10. Use hot water and rod with rubber policeman to transfer the lignin to the filter.
11. Wash the lignin with hot water to remove the acid.
12. Dry the crucible with lignin in an oven at 105±3°C to the constant weight.
13. Cool the crucible in a desiccator and weigh.



**Calculation**

Calculate the lignin content in the sample as follows:

$$\text{Lignin } (\%) = \frac{A \times 100}{W}$$

Where:

A = Weight of lignin (g)

W = Oven-dry weight of test specimen (g)

**Appendix A-11** α-cellulose (undegraded and higher-molecular-weight cellulose), β-cellulose (degraded cellulose) and γ-cellulose (hemicellulose) analysis (T-203-cm-99). (Technical Association of the Pulp and Paper Industry; TAPPI, 1999).

**Instruments**

1. Sample dispersion apparatus (Figure A-9), consisting of:
   - Variable speed motor (adjust the speed of motor to the appropriate value that no air is drawn into the sample suspension during stirring)
   - Stainless-steel stirrer with a shell

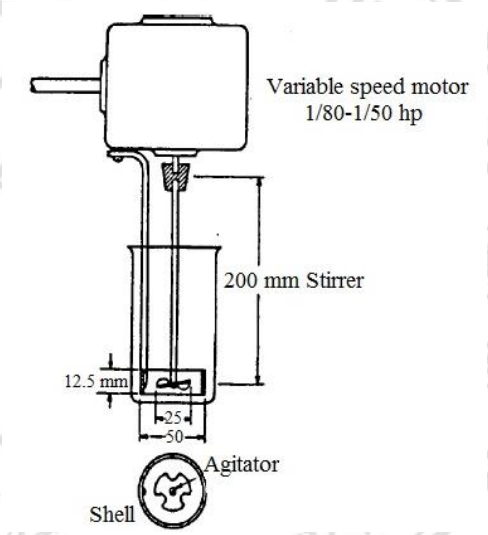


**Figure A-9** Sample dispersion apparatus. (T-203-cm-99).

**Source:** Technical Association of the Pulp and Paper Industry; TAPPI (1999)

2. Water bath
3. Filtering funnel or crucible (50 or 100 mL) with a fritted glass disk.
4. Other glassware:
   - Beaker (tall-form, 300 mL)
   - Pipettes (10, 25, 50 and 75 mL)
   - Burette (50 mL)
   - Flasks (250 and 300 mL)
   - Filtering flask (250 mL)
   - Cylinders (25, 50 and 100 mL)
   - Glass stirring rods

**Chemicals**

1. 17.5% (w/v) Sodium hydroxide (NaOH) solution
2. 0.5 N Potassium dichromate ($K_2Cr_2O_7$) solution
3. 0.1 N Ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2.6H_2O$ solution. (dissolve 40.5 g of ferrous ammonium sulfate in water, add 10 mL of concentrated sulfuric acid ($H_2SO_4$) and adjust the final volume to 1,000 mL)
4. Phenanthroline-ferrous sulfate solution. (Dissolve 1.5 g of 1,10-phenanthroline monohydrate ($C_{12}H_8N_2.H_2O$) and 0.7 g of ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) in 100 mL of water)
   (The indicator solution is also available commercially as "Ferroin")
5. 98% Sulfuric acid ($H_2SO_4$)
6. 3.0 N Sulfuric acid ($H_2SO_4$)

**Methods**

1. Allow the sample to reach moisture equilibrium in the atmosphere and weigh out two test specimens to 1.5±0.1 g.
2. Place the test specimen in a 300 mL tall-form beaker and add 75.0 mL of 17.5% (w/v) sodium hydroxide (NaOH) solution (25±0.2°C) to the beaker. Note the time at which the reagent is added.
3. Stir the reaction mixture with the sample dispersion apparatus until it is completely dispersed. Avoid drawing air into the reaction mixture during stirring.
4. Rinse the stirrer with 100 mL of 17.5% (w/v) sodium hydroxide (NaOH) solution into beaker.
5. Stir the reaction mixture and place in a water bath at 25±0.2°C for 30 min.
6. Add 100 mL of distilled water and leave the beaker in the water bath for 30 min
7. Stir the reaction mixture again with the glass rod and transfer to a filtering funnel.
8. Discard the first 10–20 mL of the filtrate, then collect 100 mL of the filtrate in a clean and dry filtration flask. (Do not rinse or wash the sample with water and do not draw air through the pulp on the filter)

**α-cellulose determination**

9. Pipette 25 mL of the filtrate and 10 mL of potassium dichromate ($K_2Cr_2O_7$) solution into the new 250 mL flask.
10. Swirl the flask and carefully add 50 mL of concentrated sulfuric acid ($H_2SO_4$).
11. Allow the solution to remain hot for 15 min.
12. Add 50 mL of water and cool down the reaction at room temperature.
13. Add 2–4 drops of phenanthroline-ferrous sulfate solution (Ferroin indicator) and titrate with 0.1 N ferrous ammonium sulfate solution to a purple color.
14. Make the blank titration by replace the sample with 12.5 mL of 17.5% (w/v) sodium hydroxide (NaOH) and 12.5 mL of water.

**β- and γ-cellulose determination**

15. Pipette 50 mL of the filtrate into a 100 mL cylinder and close the cylinder with ground glass stopper.
16. Add 50 mL of 3.0 N sulfuric acid ($H_2SO_4$) and mix by inverting.
17. Heat the cylinder in a water bath at 70–90°C for a few min to coagulate the β-cellulose.
18. Allow the precipitate until settle, overnight.
19. Gradually pour or filter the reaction mixture to obtain the clear solution.
20. Pipette 50 mL of the clear solution, 10 mL of potassium dichromate ($K_2Cr_2O_7$) and carefully add 90 mL of concentrated sulfuric acid ($H_2SO_4$) into new 300-mL flask.
21. Allow the solution to remain hot for 15 min, then proceed with titration as outlined in α-cellulose determination step.
22. Make the blank titration by replace the sample with 12.5 mL of 17.5% (w/v) sodium hydroxide (NaOH) and 12.5 mL of water and 25 mL of 3.0 N sulfuric acid ($H_2SO_4$).

**Calculations**

Calculate the α-cellulose content in sample as follow:

$$\alpha\text{ - cellulose }(\%) = 100 - \frac{6.85\,(V_1 - V_2) \times N \times 20}{A \times W}$$

Where:

$V_1$ = Titration of the sample filtrate (mL)

$V_2$ = Blank titration (mL)

N = Exact normality of the ferrous ammonium sulfate solution

A = Volume of the sample filtrate used in the oxidation (mL)

W = Oven-dry weight of sample (g)

Calculate the γ-cellulose content in sample as follow:

$$\gamma\text{ - cellulose }(\%) = 100 - \frac{6.85\,(V_4 - V_3) \times N \times 20}{25 \times W}$$

$V_3$ = Titration of the solution after precipitation of β-cellulose (mL)

$V_4$ = Blank titration (mL)

Calculate the β-cellulose content in sample:

$$\beta\text{-cellulose }(\%) = 100 - (\alpha - \text{cellulose }(\%) + \gamma - \text{cellulose }(\%))$$

**Appendix A-12** Ash in wood (T 211 om-02) (Technical Association of the Pulp and Paper Industry; TAPPI, 2002)



**Instruments**

1. Crucibles (platinum, porcelain or silica; 50 to 100 mL) with covers.
2. Electric muffle furnace (maintaining a temperature of 525±25°C).

**Methods**

1. Weigh out two test specimens at least 1.0±0.1 g (moisture-free).
2. Ignite empty crucible in a muffle furnace at 525±25°C for 30–60 min.
3. Cool crucible and place in desiccator.

4. Weigh the ignited crucible, transfer sample to crucible and close with lid.
5. Gently carbonize the sample in the crucible (without lid) on the furnace by placing the crucible in a furnace at 100°C.
6. Slowly raise the temperature to 525°C so that the sample becomes carbonized without flaming. Sample must be charred, and the temperature of the sample does not exceed 525°C.
7. Place the crucible with sample into the furnace at 525±25°C and remove the lid after the crucible seems to reach the temperature of the furnace.
8. When the specimen is completely combusted as indicated by the absence of black particles, remove the crucible from the furnace, replace the cover and allow to cool at room temperature in a desiccator.
9. Weigh the crucible with ash.
10. Repeat the ignition and weighing until the weight of the ash is constant to ±0.2 mg.

Calculate the ash content as follows:

$$\text{Ash}\,(\%) = \frac{A \times 100}{B}$$

Where

A = Weight of ash (g)

B = Weight of test specimen (g moisture-free)

# APPENDIX B

## Media

**Appendix B-1** Yeast malt-extract medium (YM) (per Liter)

| | | |
|---|---|---|
| Yeast extract | 4.0 | g |
| Malt extract | 10.0 | g |
| Glucose | 4.0 | g |

Mix all components of yeast-malt extract medium, adjust to a final volume of 1,000 mL with distilled water and then, autoclave at 121°C for 15 min.

**Appendix B-2** Basal medium supplemented with rice straw (per Liter) (Chaiyaso et al., 2011)

| | | |
|---|---|---|
| Yeast extract | 5.42 | g |
| Di-potassium hydrogen phosphate ($K_2HPO_4$) | 1.0 | g |
| Potassium di-hydrogen phosphate ($KH_2PO_4$) | 0.5 | g |
| Ammonium sulfate ($(NH_4)_2SO_4$) | 1.0 | g |
| Sodium chloride (NaCl) | 0.2 | g |
| Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) | 0.1 | g |
| Calcium chloride dihydrate ($CaCl_2.2H_2O$) | 0.1 | g |
| Tween 80 | 0.1 | g |
| Rice straw | 27.45 | g |

Mix all components of basal medium and adjust to a final volume of 1,000 mL with distilled water. The initial pH is adjusted to 7.11 with 1.0 N NaOH or 1.0 N HCl and then, autoclave at 121ºC for 20 min.

**Appendix B-3** De Man, Rogosa and Sharpe Medium (MRS) (per Liter) (Chapla et al., 2012)

| | | |
|---|---|---|
| Bacto peptone | 10.0 | g |
| Beef extract | 10.0 | g |
| Yeast extract | 5.0 | g |
| Tween 80 | 1.0 | g |
| Diammonium hydrogen citrate | 2.0 | g |
| Sodium acetate anhydrous ($CH_3COONa$) | 5.0 | g |
| Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) | 0.1 | g |
| Manganese sulphate monohydrate ($MnSO_4.H_2O$) | 0.05 | g |
| Di-potassium hydrogen phosphate ($K_2HPO_4$) | 2.0 | g |
| Supplemented either with glucose or xylose or commercial XOs or corncob-XOs or inulin or maltodextrin | 20.0 | g |

Mix all components of medium and adjust to a final volume of 1,000 mL with distilled water. The initial pH is adjusted to 6.00 with 1.0 N NaOH or 1.0 N HCl and then, autoclave at 110ºC for 20 min.

**Appendix B-4** Minimal medium for ethanol production (per Liter)

**Minimal medium for separate hydrolysis and fermentation (SHF)**

The cellulose-rich corncob hydrolysate (The initial pH was 5.0) was supplemented with

| | | |
|---|---|---|
| Ammonium sulfate ($(NH_4)_2SO_4$) | 4.0 | g |
| Yeast extract | 1.0 | g |
| Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) | 1.0 | g |
| Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) | 0.1 | g |
| Potassium metabisulfite ($K_2S_2O_5$) | 200 | ppm |

Mix all components, adjust to a final volume of 1,000 mL with cellulose-rich corncob hydrolysate and then, incubate at 30°C for 24 h before inoculation.

**Minimal medium for simultaneous saccharification and fermentation (SSF)**

| | | |
|---|---|---|
| Cellulose-rich corncob | 78 or 117 or 156 | g |
| Ammonium sulfate ($(NH_4)_2SO_4$) | 4.0 | g |
| Yeast extract | 1.0 | g |
| Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) | 1.0 | g |
| Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) | 0.1 | g |

Mix all components, adjust to a final volume of 1,000 mL with 0.1 M sodium-citrate buffer pH 5.0 and then, autoclave at 121°C for 20 min.

# APPENDIX C

## Nucleotide Sequence of 26S rRNA Gene of *Candida glabrata* KY618709

**Accession number:** KY618709

**Identify:** *Candida glabrata*

**26S rRNA sequence (660bp):**

GAATTCGAATTCGATTATCAATAAGCGGAGGAAAAGAAACCAACTGGGATT
GCCTTAGTAACGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATCTGGTA
CCTTTGGTGCCCGAGTTGTAATTTGGAGAGTACCACTTTGGGACTGTACTTT
GCCTATGTTCCTTGGAACAGGACGTCATGGAGGGTGAGAATCCCGTGTGGC
GAGGGTGTCAGTTCTTTGTAAAGGGTGCTCGAAGAGTCGAGTTGTTTGGGA
ATGCAGCTCTAAGTGGGTGGTAAATTCCATCTAAAGCTAAATACAGGCGAG
AGACCGATAGCGAACAAGTACAGTGATGGAAAGATGAAAAGAACTTTGAA
AAGAGAGTGAAAAAGTACGTGAAATTGTTGAAAGGGAAGGGCATTTGATC
AGACATGGTGTTTTGCGCCCCTTGCCTCTCGTGGGCTTGGGACTCTCGCAGC
TCACTGGGCCAGCATCGGTTTTGGCGGCCGGAAAAAACCTAGGGAATGTGG
CTCTGCGCCTCGGTGTAGAGTGTTATAGCCCTGGGGAATACGGCCAGCCGG
GACCGAGGACTGCGATACTTGTTATCTAGGATGCTGGCATAATGGTTATATG
CCGCCCGTCTTGAAACACGGACCAAGGAGTAAWTCACTAGTGAATTC

## CURRICULUM VITAE

**Author's Name** Miss Pinpanit Boonchuay

**Date of Birth** 15 September 1988

**Place of Birth** Lampang Province, Thailand

**Education**

**2006** High school from Bunyawat Witthayalai School, Lampang, Thailand

**2010** B.Sc. (Biology), Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

**Scholarships**

| Duration of Scholarship | Granter |
|---|---|
| 2011–2012 | Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand |
| 2013–2014 | Graduate School, Chiang Mai University, Chiang Mai, Thailand. |
| March–May 2016 | Student Exchange Support Program, Japan Student Services Organization (JASSO), Japan. |
| 2016-2018 | The National Research Council of Thailand (NRCT), Thailand. |

**Publications**

[1] **Boonchuay, P.,** and Chaiyaso, T. 2012. Enzymatic production of xylooligosaccharides from corncob using endo-xylanase from *Streptomyces thermovulgaris* TISTR1948. 1st Mae Fah Luang University International Conference 2012. 1-9. (Proceeding)

[2] **Boonchuay, P.**, Techapun, C., Seesuriyachan, P., and Chaiyaso, T. 2014. Production of xylooligosaccharides from corncob using a crude thermostable endo-xylanase from *Streptomyces thermovulgaris* TISTR1948 and prebiotic properties. Food. Sci. Biotechnol. 23(5): 1515-1523.

[3] **Boonchuay, P.**, Takenaka, S., Kuntiya, A., Techapun, C., Leksawasdi, N., Seesuriyachan, P., and Chaiyaso, T. 2016. Purification, characterization, and molecular cloning of the xylanase from *Streptomyces thermovulgaris* TISTR1948 and its application to xylooligosaccharide production. J. Mol. Catal. B. Enzyme. 129: 61-68.

[4] **Boonchuay, P.**, Kuntiya, A., Techanpun, C., Leksawasdi, N., Seesuriyachan, P., Hanmoungjai, P., Watanabe, M., Takenaka, S., and Chaiyaso, T. 2017. Optimization of fermentable sugar production from cellulose-rich corncob residue a waste from xylooligosacharides production process. The 29th Annual Meeting of the Thai Society for Biotechnology and International Conference. 148-160. (Proceeding)



[5] **Boonchuay, P.**, Techapun, C., Leksawasdi, N., Seesuriyachan, P., Hanmoungjai, P., Watanabe, M., Takenaka, S., and Chaiyaso, T. 2018. An integrated process for xylooligosaccharide and bioethanol production from corncob. Bioresour. Technol. 256: 399-407.

**Presentations**

[1] **Boonchuay, P.,** Techapun, C., Hanmoungjai, P., and Chaiyaso, T. 2012. Enzymatic production of xylooligosaccharide (XO) from selected agricultural wastes. International Conference on Food and Applied Bioscince. February 6th–7th, 2012, Kantary Hills Hotel, Chiang Mai, Thailand. (Poster Presentation)

[2] **Boonchuay, P.** and Chaiyaso, T. 2012. Enzymatic production of xylooligosaccharides from corncob using endo-xylanase from *Streptomyces thermovulgaris* TISTR1948. 1st Mae Fah Luang University International Conference 2012. November 29th–December 1st, 2012. Mae Fah Luang University, Chiang Rai, Thailand. (Oral Presentation)

[3] **Boonchuay, P.**, Kuntiya, A., Techanpun, C., Leksawasdi, N., Seesuriyachan, P., Hanmoungjai, P., and Chaiyaso, T. 2014. Production of xylooligosaccharides (XOs) from agricultural wastes by thermostable endo-xylanase from *Streptomyces thermovulgaris* TISTR1948 and theirs prebiotic properties. The 3rd Thailand Nation Reseach University Summit (ThaiNRU III). July 31st–August 1st, 2014. Bangkok Convention Centre, Centara Grand, Central World, Bangkok, Thailand. (Poster Presentation)

[4] **Boonchuay, P.**, Takenaka, S., Watanabe, M., Kuntiya, A., Techanpun, C., Leksawasdi, N., Seesuriyachan, P., Hanmoungjai, P., and Chaiyaso, T. 2015. Purification and characterization of thermostable cellulase-free endo-xylanase from *Streptomyces thermovulgaris* TISTR1948. July 29th–31th, 2015. The 6th International Conference on Fermentation Technology for Value Added Agricultural Products, Centara Hotel and Convention Centre, Khon Kaen, Thailand. (Oral Presentation)

[5] **Boonchuay, P.**, Takenaka, S., Watanabe, M., Kuntiya, A., Techanpun, C., Leksawasdi, N., Seesuriyachan, P., Hanmoungjai, P., and Chaiyaso, T. 2016. Purification and characterization of

thermostable cellulase-free endo-xylanase from *Streptomyces thermovulgaris* TISTR1948 and its application on xylooligosaccharide production. February 4th–5th, 2016. International Conference on Food and Applied Bioscience 2016. The Empress Hotel, Chiang Mai, Thailand. (Poster Presentation)

[6] **Boonchuay, P.**, Kuntiya, A., Techanpun, C., Leksawasdi, N., Seesuriyachan, P., Hanmoungjai, P., Watanabe, M., Takenaka, S., and Chaiyaso, T. 2017. Optimization of fermentable sugar production from cellulose-rich corncob residue a waste from xylooligosacharides production process. November 23rd–25th, 2017. The 29th Annual Meeting of the Thai Society for Biotechnology and International Conference. Swissôtel Le Concorde, Bangkok, Thailand. (Poster Presentation)