Appendices

Appendix 1 Composition and preparation of reagents for micro-kjeldahl method

Sodium hydroxide- Sodium thiosulphate solution: Dissolve 600g of NaOH and 50g of Na₂SO₃.5H₂O in water and make up to 1 liter.

4% Boric acid solution: Dissolve 4g of H₃BO₃ in warm water and dilute to 100ml.

Mixed indicator solution: Mix 2 parts of 0.2% methyl red in ethanol with 1 part of 0.2% methylene blue in ethanol

Appendix 2 Composition and preparation of reagents for phenol sulphuric acid method

5% phenol: Dissolve 50g of redistilled (reagent grade) phenol in water and dilute to 1 litre.

96% Sulphuric acid (reagent grade)

Standard glucose: Stock – 100mg in 100ml of distilled water.

Working standard-10ml of stock diluted to 100ml with distilled water.

Appendix 3 Composition and preparation of reagents for DNS method

DNS reagent: Dissolve simultaneously 1g of DNS, 200mg of crystalline phenol and 50mg of sodium sulphite in 100ml of 1%NaOH solution by stirring. Store the reagent in a stoppered bottle at 4°C.

Standard glucose solution: 100mg in 100ml distilled water.

Appendix 4 Composition and preparation of reagents for Lowry’s method

Solution A: 2% Sodium carbonate in 0.1N NaOH

Solution B: 0.5% CuSO₄ in 1% Sodium Potassium tartrate
Studies on ethanol production from bioconverted agricultural residues

**Solution C:** Mix 50ml of Solution A with 1ml of Solution B

**FC reagent**

Stock protein: 50mg Bovine serum albumin/50ml of distilled water

**Working Std.:** Dilute 10ml of stock to 50ml (concentration 200µg/ml).

**Appendix 5 Composition of media for screening cellulytic fungi**

- CMC - 1 g
- Agar - 15 g
- NaNO₃ - 1 g
- K₂HPO₄ - 1 g
- MgSO₄·7H₂O - 0.5 g
- Yeast extract - 1 g
- Distilled water - 1000 ml

and to eliminate the bacterial contamination 8 ml of 1% streptomycin was added.

**Appendix 6 Composition of Mandle’s media (in g l⁻¹)**

- Urea 0.3; (NH₄)₂SO₄ 1.4; KH₂PO₄ 2; CaCl₂ 0.3; MgSO₄·7H₂O 0.3; Peptone 0.75 and Yeast extract 0.25; Carbon source 10. Trace elements were also added, using a 1% (v/v) solution of salts (ml l⁻¹): FeSO₄·7H₂O 0.5; MnSO₄ 0.16; ZnSO₄ 0.14; CoCl₂ 2.
- pH 5.5-6.0 before sterilization.
Appendix 7 Composition and preparation of reagents for FPU assay

DNS Reagent:
Mix distilled water 1416 ml,
3,5 Dinitrosalicylic acid 10.6 g,
Sodium hydroxide 19.8 g, Dissolve and then add:
Rochelle salt (sodium potassium tartrate) 306 g,
Phenol (melt at 50°C) 7.6 ml,
Sodium metabisulfite 8.3 g.

Citrate Buffer:

Citric acid monohydrate 210 g,
Distilled water 750 ml,
NaOH - add until pH equals 4.3 (50 to 60 g)

Dilute to 1 liter and check pH. If necessary add NaOH until the pH is 4.5. When the 1 M citrate buffer stock is diluted with distilled water to 50 mM the pH should be 4.8. After diluting the citrate buffer check and adjust the pH if necessary to pH 4.8.

Blank and controls: Reagent blank was 1.5 ml citrate buffer. Enzyme control was 1.0 ml citrate buffer and 0.5 ml enzyme dilution (separate control for each dilution tested). Substrate control was 1.5 ml citrate buffer and filter-paper strip.

Glucose standards: A working stock solution of anhydrous glucose (10 mg/ml⁻¹) should be made up. Aliquots of this working stock should be tightly sealed and stored frozen. Dilutions are made from the working stock in the following manner:

1.0 ml + 0.5 ml buffer = 1:1.5 = 6.7 mg/ml (3.35 mg/0.5 ml).
1.0 ml + 1.0 ml buffer = 1:2 = 5 mg/ml (2.5 mg/0.5 ml).
1.0 ml + 2.0 ml buffer = 1:3 = 3.3 mg/ml (1.65 mg/0.5 ml).
1.0 ml + 4.0 ml buffer = 1:5 = 2 mg/ml (1.0 mg/0.5 ml).
Appendix 8 Composition and preparation of reagents for CMCase assay

**Substrate:** 2% Carboxymethyl cellulose CMC in 0.05 M sodium citrate buffer, pH 4.8.

**DNS Reagent:** same as appendix 8

**Citrate Buffer:** same as appendix 8

**Glucose standards:**

2 mg ml$^{-1}$ anhydrous glucose

Aliquots of about 5 ml can be stored frozen

Stir well after thawing

Undiluted = 2.0 mg ml$^{-1}$ (1.0 mg/0.5 ml)

1.0 ml + 0.5 ml buffer = 1:1.5 = 1.33 mg ml$^{-1}$ (0.67 mg/0.5 ml)

1.0 ml + 1.0 ml buffer = 1:2 = 1.0 mg ml$^{-1}$ (0.5 mg/0.5 ml)

1.0 ml + 3.0 ml buffer = 1:4 = 0.5 mg ml$^{-1}$ (0.25 mg/0.5 ml)

Appendix 9 Composition of media for xylanase production (in g l$^{-1}$)

NaNO$_3$, 3.0; KCl, 0.5; KH$_2$PO$_4$, 1.0; MnSO$_4$.7H$_2$O, 0.5; FeSO$_4$.7H$_2$O, 0.01; and Oat spelt Xylan, 10. One liter of the media was supplemented with 1.0 ml of trace solution containing ZnSO$_4$, 1.0 g l$^{-1}$ and CuSO$_4$.5H$_2$O, 0.5 g l$^{-1}$. pH - 5.6

Appendix 10 Composition and preparation of reagents for xylanase assay

**Xylan substrate solution (1.0%):**

Accurately weigh 1.0 g xylan (dry base, from oat spelt), transfer to a beaker with 60 ml of 0.2 M acetate buffer (pH 4.5). Stir for 30 min and incubate at 60ºC for 1 hr with gradually stirring and check pH (4.5). Transfer the solution into a 100 ml volumetric flask and make up to volume with distilled water.
3,5-Dinitrosalicylic acid (DNS) solution:

Accurately weigh 10 g of DNS into a 2000 ml beaker. Add 16 g of sodium hydroxide pellets, 300 g of potassium sodium tartrate and 500 ml of distilled water. Place the beaker on a heater/stirrer and warm gently, whilst stirring, to dissolve. Cool to ambient temperature and transfer the contents of the beaker into a 1000 ml volumetric flask. Rinse the beaker with distilled water; add rinsings to the volumetric flask and make up to volume with distilled water.

Xylose standard dilutions:

Accurately weigh 0.5g of anhydrous xylose with distilled water and make up to 100 ml in a volumetric flask. Dilute with distilled water to get working standard solutions containing 250, 500 and 750 μmoles⁻¹ of xylose.

Appendix 11 Composition of media for laccase production (in g/l)

Lignin, 10; Glucose 40; Glycerol 7; L histidine, 0.50; CuSO₄, 0.10; NaNO₃, 1.80; NaCl, 0.180; KCl, 0.50; CaCl₂.H₂O, 0.50; FeSO₄.7H₂O, 0.05; KH₂PO₄.1; MgSO₄.7H₂O, 0.50.

Appendix 12 Preparation of reaction mixture for laccase assay

The reaction mixture contained 50μl crude supernatant, 50μl of 5mM ABTS in 25mM sodium succinate buffer pH 5 and 900μl deionised water. The enzyme blank consisted of 50μl crude supernatant, 50μl 25mM sodium succinate buffer pH 5 and 900 μl deionised water and substrate blank consisted of 50μl of 5mM ABTS in 25mM sodium succinate buffer pH 5 and 950μl deionised water.
Appendix 13 Composition and preparation of reagents for PAGE

Separating gel buffer: 1 M Tris/ HCl pH 8.8 -18.16g Tris dissolved in 80ml distilled water. pH is adjusted to 8.8 with concentrated HCl. Volume is made up to 100ml with distilled water after adjustment of pH.

Stacking gel buffer: 1 M Tris/HCl pH 6.8 – 3g Tris dissolved in 30ml distilled water. The pH is adjusted to 6.6 with concentrated HCl and then volume is made up to 50ml with distilled water.

CMC (1 %) solution in distilled water.

Acrylamide / bisacrylamide (40 %): 38.96g acrylamide and 1.04g bisacrylamide dissolved in 100ml of distilled water.

Sodium dodecyl sulfate (SDS; 10 %)

Ammonium persulfate (APS; 10 %)

Sample buffer: 300 µl distilled water, 300 µl of 1 % SDS, 125 µl stacking gel buffer, 200 µl 50 % glycerol, 50 µl 0.05 % 2-mercaptoethanol and 300 µl of 0.1% bromophenol blue.

Staining solution: 0.5g of coomassie brilliant blue dissolved in mixture of 100ml distilled water, 20ml glacial acetic acid, 80ml methanol(5:1:4).

Destaining solution: A mixture of 100ml distilled water, 20ml glacial acetic acid, 80ml methanol (5:1:4).
### Resolving gel

<table>
<thead>
<tr>
<th>Solution</th>
<th>Gel for enzyme determination (Zymogram)</th>
<th>Gel for protein determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>0.75 ml</td>
<td>1.95 ml</td>
</tr>
<tr>
<td>Separating gel buffer</td>
<td>2.26 ml</td>
<td>2.26 ml</td>
</tr>
<tr>
<td>CMC (1 %)</td>
<td>1.2 ml</td>
<td>0.0 ml</td>
</tr>
<tr>
<td>Acrylamide (40 %)</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
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<tr>
<td>SDS (10 %)</td>
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<td>60 µl</td>
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<tr>
<td>APS (10 %)</td>
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<td>48 µl</td>
</tr>
<tr>
<td>TEMED</td>
<td>5 µl</td>
<td>5 µl</td>
</tr>
</tbody>
</table>

### Stacking gel

- Distilled water 3.7 ml
- Stacking gel buffer 625 µl
- Acrylamide (40 %) 625 µl
- SDS (10 %) 50 µl
- TEMED 5 µl
- APS (10 %) 50 µl

### Appendix 14 Composition of YPD media (in g l⁻¹)

Bacto Yeast Extract, 10 g l⁻¹; Bacto Peptone, 10 g l⁻¹; D-glucose, 50 g l⁻¹

### Appendix 15 Composition of media for SSF (in g l⁻¹)

Yeast extract, 5; (NH₄)₂SO₄, 7.5; K₂HPO₄, 3.5; MgSO₄·7H₂O, 0.75; CaCl₂·2H₂O, 1; Pretreated substrate, 10.
Appendix 16 Composition and preparation of reagents for Spectrophotometric determination of ethanol

Standard ethanol: Standard stock of 100% pure analytical grade (containing 789mgml⁻¹) ethanol was prepared by dissolving 12.6ml of ethanol in 100ml distilled water which results in 100mgml⁻¹.

0.23N Potassium dichromate solution: 34g of potassium dichromate was dissolved in 500ml distilled water and 325ml of concentrated sulphuric acid was added and the volume was made up to 1000ml with distilled water.

Appendix 17 Inoculum preparation of fungi for SSF in Fermenter

The inocula were prepared using media containing (gl⁻¹): Glucose, 50; Yeast extract, 5; (NH₄)SO₄, 7.5; K₂HPO₄, 3.5; MgSO₄·7H₂O, 0.75; CaCl₂·2H₂O, 1 and 0.05M buffer citrate at pH 5.5.

Volumes of 50 ml media were sterilized and inoculated with respective fungus in 250 ml cotton-plugged Erlenmeyer flasks, and then incubated for 72 h at 30°C in an incubator shaker (150 rpm). At the end of incubation, the contents of these flasks were aseptically centrifuged and used for SSF. It resulted in inoculation of SSF by 1.0g biomass (based on the dry biomass).