# CONTENTS

1.0 INTRODUCTION 1-5

2.0 REVIEW OF LITERATURE 6-50
   2.1 Composition of Lignocellulosic 6
      2.1.1 Cellulose 7
      2.1.2 Hemicellulose 8
      2.1.3 Lignin 9
   2.2 Cellulase Producing Microorganisms 10
      2.2.1 Occurrence 10
   2.3 Metagenomics for Isolating Novel Glycosyl Hydrolases from Uncultural Bacteria 14
   2.4 Cellulase Activity Assays 16
   2.5 Cellulases 21
   2.6 Cellulosome 22
   2.7 Classification of Cellulase 23
   2.8 Secretome Analysis of Glycosyl Hydrolases 26
   2.9 Multiplicity 29
   2.10 Regulation 30
   2.11 Purification of Cellulases 33
   2.12 Production of Cellulase under Solid State Fermentation 39
      2.12.1 Substrate (Carbon Source) 40
      2.12.2 Nitrogen Source 41
      2.12.3 Moisture Content 42
      2.12.4 Temperature and pH 43
      2.12.5 Effect of Additives 43
      2.12.6 Effect of Inoculum 44
      2.12.7 Optimization using Statistical Designs 44
   2.13 Application of Cellulases 45
      2.13.1 Cellulases in Bio-de-inking 46
      2.13.2 Cellulases in ethanol production 47

3.0 MATERIALS AND METHODS 51-69
   Chemicals 51
   3.1 Isolation of Fungal Cultures 51
3.2 Culture

3.3 Morphology and Identification
  3.3.1 Slide Culture
  3.3.2 Extraction of DNA
  3.3.3 PCR Amplification

3.4 Solid Substrate Culturing for Enzyme Production
  3.4.1 Inoculum development
  3.4.2 Culture conditions

3.5 Deinking Experiments

3.6 Optimization of Culture Conditions for Enzyme Production by *Aspergillus fumigatus* (AMA)
  3.6.1 Effect of Different Carbon Sources
  3.6.2 Effect of Media Types
  3.6.3 Effect of Nitrogen Sources
  3.6.4 Effect of Nitrogen Concentration
  3.6.5 Effect of Initial pH of Medium
  3.6.6 Effect of Temperature
  3.6.7 Effect of Initial Moisture Level
  3.6.8 Effect of Inoculum Age
  3.6.9 Effect of Different Additives
  3.6.10 Effect of Different Tween-80 concentration on enzyme production

3.7 Statistical Model: Methodology and Design of Experiments

3.8 Regulation of Enzyme Production
  3.8.1 Induction and Repression of endoglucanase and β-glucosidase in *A. fumigatus*
  3.8.2 Native polyacrylamide gel electrophoresis and zymogram
  3.8.3 Metabolite profiling of crude endoglucanase using TLC

3.9 2D Gel Electrophoresis
  3.9.1 2-Dimensional gel electrophoresis of secretome
  3.9.2 Zymograms for detection of enzyme activities in 2DE and IEF gels
  3.9.3 Protein Identification
3.10 Purification of Endoglucanases, Cellbiohydrolase and β-glucosidases from *A. fumigatus* fresenius (AMA)

3.10.1 Ion-Exchange Chromatography
3.10.2 Phenyl Sepharose 6FF hydrophobic interaction (HIC)
3.10.3 Poly-buffer exchanger (PBE-94)
3.10.4 Gel-Filtration Chromatography

3.11 Characterization of Enzymes

3.11.1 Molecular Weight Determination by SDS-PAGE
3.11.2 Determination of pl by IEF
3.11.3 Temperature and pH Optima
3.11.4 Stability
3.11.5 Effect of metal ions and additives
3.11.6 Substrate specificity (EG, CBH and β-G)
3.11.7 Enzymes Kinetics
3.11.8 Hydrolysis product of purified endoglucanase (EG) by TLC

3.12 Enzymatic Hydrolysis and Fermentation

3.13 Estimation of Enzyme Activities

3.13.1 Endoglucanase
3.13.2 Avicel Adsorbable Endoglucanase (AAEG)
3.13.3 β-glucosidase
3.13.4 Cellbiohydrolase
3.13.5 Total Cellulase Activity (Fpase)
3.13.6 Xylanase

3.14 Estimation of Protein

3.15 Enzyme Units

4.0 RESULTS

4.1 Screening of Fungal Isolates for Production of Cellulases and Xylanase
4.2 Deinking Experiments
4.3 Culture
4.4 Optimization of Culture for Enzyme Production by *A. fumigatus*

4.4.1 Effect of Different Carbon Sources on Production of Cellulase and Xylanase
4.4.2 Effect of Different Media Types Production of Cellulase and Xylanase
4.4.3 Effect of Nitrogen Sources Production of Cellulase and Xylanase
4.4.4 Effect of Different Concentration of beef extract on Production of Cellulase and Xylanase
4.4.5 Effect of Different Concentration of Ammonium Sulphate on Production of Cellulase and Xylanase
4.4.6 Effect of Different pH on Production of Cellulase and Xylanase
4.4.7 Effect of Different Temperature on Production of Cellulase and Xylanase
4.4.8 Effect of Moisture Level on Production of Cellulase and Xylanase
4.4.9 Effect of Different Inoculum Age on Production of Cellulase and Xylanase
4.4.10 Effect of Different Additives on Production of Cellulase and Xylanase
4.4.11 Effect of Different Concentration of Tween-80 on Cellulase and Xylanase

4.5 Box-Behnken Design of Experiments for Cellulase and Xylanase by A. fumigatus

4.5.1 Endoglucanase (EG)
4.5.2 β-glucosidase (β-G)
4.5.3 Filter Paper Activity (Fpase)
4.5.4 Cellobiohydrolase (CBH)
4.5.5 Xylanase

4.6 Regulation of Cellulase and Xylanase production A. fumigatus

4.6.1 Induction of Cellulase and Xylanase
  4.6.1.1 Induction of Endoglucanase
  4.6.1.2 Induction of β-glucosidase
  4.6.1.3 Induction of Cellobiohydrolase
  4.6.1.4 Induction of Xylanase
4.6.2 Repression of Cellulase and Xylanase
  4.6.2.1 Regulation of Endoglucanase Activity
  4.6.2.2 Regulation of β-glucosidase Activity
4.6.2.3 Regulation of Cellobiohydrolase Activity 92
4.6.2.4 Regulation of Xylanase Activity 93
4.6.3 Production Profile and Sequential Induction of EG and β-G 93
4.6.3.1 Metabolic Footprinting during EG Production 93
4.7 Two Dimensional Electrophoretic Profiling of Secretome 94
4.8 Purification of Endoglucanase 98
4.8.1 Molecular Weight and pI of Purified EG 99
4.8.2 Temperature, pH Optima and Stability 99
4.8.3 Effect of Metal Ions and Chemical 100
4.8.4 Substrate Specificity of Endoglucanase (EG) 100
4.8.5 Kinetics 100
4.8.6 Hydrolyses Studies 102
4.9 Purification of Cellobiohydrolase (CBH) 102
4.9.1 Molecular Weight and pI of Purified CBH 103
4.9.2 Temperature, pH Optima and Stability 103
4.9.3 Effect of Metal Ions and Chemical 104
4.9.4 Substrate Specificity of Cellobiohydrolase (CBH) 104
4.9.5 Effect of Monosaccharides/Disaccharides on activity of CBH 104
4.9.6 Kinetics 104
4.10 Purification of β-glucosidase 106
4.10.1 Molecular Weight and pI of Purified β-G 106
4.10.2 Temperature, pH Optima and Stability 107
4.10.3 Effect of Metal Ions and Chemical 107
4.10.4 Substrate Specificity of β-G 107
4.10.5 Effect of Monosaccharides/Disaccharides on activity 107
4.10.6 Kinetics 107
4.11 Hydrolysis and Ethanol Production from Alkali-Treated Rice Straw 109

5.0 DISCUSSION 110-127
6.0 SUMMARY 128-136
7.0 REFERENCES 137-189
Appendix  i-iv
List of Publications