Chapter-1: Introduction

1.1. Objectives.

Chapter-2: Review of Literature

2.1. Physico-Chemical Parameters

2.2. Process Economization of $\alpha$-Amylase production using Basal Medium

2.2.1. Influence of Carbon Sources

2.2.2. Influence of Peptone and other N$_2$ sources

2.2.3. Influence of Metals on $\alpha$-Amylase Production

2.2.4. Influence of C:N Ratio on $\alpha$-Amylase Production

2.2.5. Bacterial and Fungal Immobilization

2.2.6. Use of Agro-wastes for $\alpha$-Amylase Production

2.3. Purification and Characteristics of $\alpha$-Amylase

2.4. Influence of Immobilization on Bacterial $\alpha$-Amylase

2.5. Applications of $\alpha$-Amylase

3.1. Isolation and Screening of Bacterial Species/Strains

3.1.1. Collection of Soil Samples

3.1.2. Screening of Potent $\alpha$-Amylase Producing Soil Bacteria

3.1.3. Primary Screening for $\alpha$-Amylase Producers

3.1.4. Secondary Screening for $\alpha$-Amylase Producers

3.2. Bacterial Species/Strain Identification

3.2.1 Presumptive Identification (up to generic level) of the Bacterial Species/Strain

3.2.2 Production of $\alpha$-Amylase in Batch Cultures (Submerged Fermentation)

3.2.2.1 Cultivation Medium

3.2.2.2 Preparation of the Inoculum

3.2.2.3 $\alpha$-Amylase Production through Submerged Fermentation (SmF)

3.2.2.4 $\alpha$-Amylase Assay

3.2.2.5 Identification of the Bacterium KLM14 by 16S rRNA gene sequencing and PCR amplification
3.3 Optimization of Process Variables for α-amylase production through Submerged Fermentation of the Basal Medium…………………………………………………………74
  3.3.1 Optimization of pH……………………………………………………………75
  3.3.2 Optimization of Temperature………………………………………………75
  3.3.3. Optimization of Initial Inoculum Size……………………………………75
  3.3.4. Optimization of Substrate Concentration……………………………..76

3.4. Process Economization for α-Amylase Production in Submerged Fermentation Basal Medium……………………………………………………………………76
  3.4.1. Influence Carbon sources on Enzyme Production………………………76
  3.4.2. Influence Nitrogen sources on Enzyme Production……………………76
    3.4.2.1. Influence of Peptone on α-Amylase Production……………………76
    3.4.2.2. Influence of Other Organic Nutrients on α-Amylase Production……76
  3.4.3. Influence of Inorganic Nutrients on α-Amylase Production……………76
  3.4.4. Influence of Metal Salts on α-Amylase Production……………………..78
    3.4.5.1 Influence of C:N Ratio using Peptone as N₂ Source…………………79
    3.4.5.2 Influence of C:N Ratio using Ammonium Bisulphate as N₂ Source……79
  3.4.5. Overall Influence of Nutrients on α-Amylase Production………………79
  3.4.6. Overall Influence of Nutrients on α-Amylase Production………………79
  3.4.7. Bacterial Cell Immobilization………………………………………………80
    3.4.7.1. Cultivation of B. megaterium KLMI4……………………………………80
    3.4.7.2. Immobilization in Sodium Alginate………………………………………80
  3.4.8. Use of Agro-wastes for Economization of α-Amylase Production through Submerged fermentation…………………………………………………………81
    3.4.8.1. Preparation of the Agro-wastes for fermentation……………………81
    3.4.8.2. Composition of Agro-wastes………………………………………………82
      3.4.8.2.1. Estimation of total carbohydrates………………………………………82
      3.4.8.2.2. Estimation of total protein………………………………………………82
      3.4.8.2.3. Estimation of total fats…………………………………………………82
    3.4.8.3. Preparation of the Fermentation Medium……………………………..83
    3.4.8.4. Optimization of the Process Parameters using Agro-wastes as substrate……………………………………………………………………83
      3.4.8.4.1. Optimization of pH………………………………………………………83
      3.4.8.4.2. Optimization of Temperature…………………………………………84
      3.4.8.4.3. Optimization of Initial Inoculum Size………………………84
3.4.8.5. Influence of Carbon and Nitrogen Sources on \( \alpha \)-Amylase Production

3.4.8.5.1. Influence of Carbon Sources ................................................................. 85
3.4.8.5.2. Influence Nitrogen Sources ..................................................................... 85
  3.4.8.5.2.1. Peptone Supplementation and \( \alpha \)-Amylase Production ......................... 85
  3.4.8.5.2.2. Supplementation of other Organic Nutrients ........................................ 85
3.4.8.5.3. Supplementation of Inorganic Nutrients ............................................... 86
3.4.8.5.4. Supplementation of Metal Salts .............................................................. 86
3.4.8.5.5. Overall Influence of Nutrient Supplementation on \( \alpha \)-Amylase Production ................................................................. 87

3.5. Protein Estimation ............................................................................................ 87

3.6. Enzyme Purification ......................................................................................... 88
  3.6.1. Salt precipitation .......................................................................................... 88
  3.6.2. Dialysis ...................................................................................................... 88
  3.6.3. Ion-Exchange Chromatography .................................................................. 88

3.7. Enzyme Characterization .................................................................................. 89
  3.7.1. Molecular Weight Determination ............................................................... 89
  3.7.2. Crude Enzyme Characterization ................................................................. 89
    3.7.2.1. Enzyme stability at different \( pH \) levels ............................................... 89
    3.7.2.2. Thermostability of the Crude Enzymes ............................................... 89
  3.7.3. Characterization of Purified Free and immobilized Enzyme ....................... 90
    3.7.3.1. Thermo-stability of the Enzyme .......................................................... 91
    3.7.3.2. Enzyme Stability at different \( pH \) ....................................................... 91
    3.7.3.3. Reusability of Immobilized Enzyme ................................................... 91
    3.7.3.4. Storage Stability of the Free and Immobilized Enzyme ....................... 92
    3.7.3.5. Kinetic Analysis of Free and Immobilized forms of \( \alpha \)-amylase .......... 92

3.8. Applications of \( \alpha \)-Amylase ........................................................................... 92
  3.8.1. \( \alpha \)-Amylase as Desizing Agent In Textile Industries .................................... 93
    3.8.1.1. Effect of Enzyme Concentration on the process of Desizing ............... 93
    3.8.1.2. Effect of Temperature on the process of Desizing ............................... 93
    3.8.1.3. Effect of \( pH \) on the process of Desizing ............................................ 94
    3.8.1.4 Time Profile for the Desizing of the Cloth ........................................... 94
  3.8.2. \( \alpha \)-Amylase as Additive In Detergent Formulations .................................. 94
    3.8.2.1. Residual Enzyme Activity in the presence of Detergents ..................... 94
    3.8.2.2. Evaluation of Washing Performance .................................................. 95
Chapter 4: Results

4.1. Screening Soil Samples for α-Amylase Producing Bacteria

4.2. Identification of the Bacterial Strain (up to Generic Level)

4.3. Identification of the Strain KLMI4 by 16s rRNA Gene Sequencing

4.4. Optimization of Process Parameters of Submerged Fermentation (Basal Medium)

4.4.1. Optimization of pH

4.4.2. Optimization of temperature

4.4.3. Optimization of inoculum size

4.4.4. Optimization of substrate concentration

4.5. Process Economization using Basal Medium

4.5.1. Influence of Carbon Sources

4.5.2. Influence of Organic Nitrogen Sources

4.5.2.1. Influence of Peptone

4.5.2.2 Influence of other Organic Nitrogen Sources

4.5.3. Influence of Inorganic Nitrogen Sources

4.5.4. Influence of some Metal Salts on Enzyme Production

4.5.5. Influence of C:N Ratio in Fermenting Medium on α-Amylase Production

4.5.6. Influence of Combination of all Optimized Variable Parameters

4.6. Process Economization through Whole Cell Immobilization

4.6.1. Whole Cell Immobilization

4.6.2. Reusability of the Immobilized Cells

4.7. Process Economization by using Agro-wastes as Substrate

4.7.1. Biochemical Evaluation of the Agro-wastes

4.7.2. Optimization of Basic Variable Process Parameters

4.7.2.1. Optimization of substrate concentration and selection of the substrate

4.7.2.1.1. Rice husk extract

4.7.2.1.2 Groundnut oil cake extract

4.7.2.1.3 Green gram husk extract

4.7.2.1.4 Selection of the substrate with maximum potential for α-amylase production

4.7.2.2. Optimization of pH

4.7.2.3. Optimization of Temperature
4.7.2.4. Optimization of inoculum size ............................................... 121
4.7.3. Process Economization using Green Gram Husk ......................... 121
  4.7.3.1. Influence of C sources ..................................................... 121
  4.7.3.2. Influence of Nitrogen Sources on Enzyme Production ............. 122
    4.7.3.2.1. Influence of Peptone on Enzyme Production .................. 123
    4.7.3.2.2. Influence of organic N sources on enzyme production ... 123
    4.7.3.2.3. Influence of inorganic nitrogen sources on enzyme production ................................................ 124
  4.7.3.3. Influence of some Metal Salts on Enzyme Production .............. 124
  4.7.3.4. Influence of Combination of all Optimized Variables on α-Amylase Activity ...................................................................... 125
4.8. Enzyme Purification and Protein Estimation .................................. 126
  4.8.1 Crude Enzyme Protein Extraction ........................................... 126
  4.8.2 Enzyme Purification ............................................................ 126
4.9. Characterization of Enzyme ....................................................... 127
  4.9.1. Molecular Weight Determination .......................................... 127
  4.9.2. Crude Enzyme Characterization ............................................. 127
    4.9.2.1. Crude Enzyme Stability at different pH conditions .......... 127
    4.9.2.2. Thermo-stability of the Crude Enzyme ............................. 128
  4.9.3. Characterization of Purified Free and Immobilized Enzyme ........ 128
    4.9.3.1. Estimation of Enzyme Immobilized in the Alginate Beads ...... 128
    4.9.3.2. Physico-chemical Properties of the Free and Immobilized Enzyme  ............................................................ 129
      4.9.3.2.1. Thermo-stability of free and immobilized α-amylase 129
      4.9.3.2.2. Influence of pH on free and immobilized enzyme ....... 130
    4.9.3.3. Reusability of Immobilized Enzyme .................................. 131
    4.9.3.4. Storage stability of the Free and Immobilized α-amylase .... 131
    4.9.3.5. Kinetic Analysis of Free and Immobilized forms of α-amylase 132
      4.9.3.5.1 Kinetic Analysis of Free Enzyme ................................ 133
      4.9.3.5.2 Kinetic Analysis of Immobilized Enzyme ............... 134
4.10. Applications of α-Amylase ...................................................... 134
  4.10.1. α-Amylase as Desizing Agent in Textile Industries .................. 134
    4.10.1.1. Effect of enzyme concentration on the process of Desizing .... 134
    4.10.1.2. Effect of Temperature on the process of Desizing .......... 135
4.10.1.3. Effect of pH on the process of Desizing…………………………..135
4.10.1.4. Time Profile for the Desizing of the Cloth…………………………..136
4.10.2. α-Amylase as Additive in Detergent Formulations……………………..136
4.10.2.1. Residual Enzyme Activity in the presence of Detergents………..136
4.10.2.2. Evaluation of Washing Performance…………………………..138

Chapter-5: Discussion..........................................................................................142-160
5.1. Isolation, Screening and Identification.....................................................................................142
5.2. Basic Variable Parameters of Fermentation Process.........................................................142
  5.2.1. Optimization of pH.......................................................................................143
  5.2.2. Optimization of Temperature.........................................................................143
  5.2.3. Optimization of Inoculum Size.........................................................................144
  5.2.4. Optimization of Substrate Concentration..................................................144
5.3. Process Economization of A-Amylase Production.........................................................146
  5.3.1. Process Economization Using Basal Medium...........................................146
    5.3.1.1. Influence of Carbon Sources..............................................................146
    5.3.1.2. Influence of Organic N$_2$ Sources.....................................................147
    5.3.1.3. Influence of Inorganic N$_2$ Sources...................................................148
    5.3.1.4. Influence of Metal Salts........................................................................149
    5.3.1.5. Influence of C: N Ratio on A-Amylase Production..........................150
  5.3.2. Process Economization through Bacterial Whole Cell Immobilization...........152
  5.3.3. Process Economization Using Agro-Wastes.............................................152
5.4. Enzyme Purification............................................................................................154
5.5. Enzyme Characterization through Immobilization..................................................154
5.6. Applications of α-Amylase......................................................................................158
  5.6.1. α-Amylase as Desizer................................................................................158
  5.6.2. α-Amylase as Additive In Detergent Formulations......................................160

Chapter-6: Summary and Conclusions............................................................................161-169

Chapter-7: References.................................................................................................170-209

Publication