
TABLE OF CONTENTS

| | Page No. |
|--|-----------------|
| List of Figures | i-iv |
| List of Tables | v-vi |
| Symbols Used | vii-viii |
| Abbreviations Used | ix |
| Preface | x-xi |
| Chapter 1 Introduction | 1-12 |
| 1.1 Immunosuppressants | 1 |
| 1.2 Approval of rapamycin as immunosuppressant | 4 |
| 1.3 Rapamycin as compared with other immunosuppressants | 5 |
| 1.4 Other roles of rapamycin | 6 |
| 1.4.1 Treatment of tuberous sclerosis | 8 |
| 1.4.2 Reduction in occurrence of skin cancer | 8 |
| 1.4.3 Treatment of Psoriasis | 8 |
| 1.4.4 Anti-tumor effect | 8 |
| 1.4.5 Anti-ageing property | 9 |
| 1.4.6 Potent role against Human Immunodeficiency Virus (HIV) | 9 |
| 1.5 Use of rapamycin in coronary stents | 10 |
| 1.6 Nano-rapamycin | 10 |
| 1.7 Side effects associated with rapamycin | 11 |
| 1.8 Manufacturers of rapamycin | 11 |
| Chapter 2 Review of Literature and Objectives | 13- 45 |
| 2.1 Rapamycin | 13 |

| | | |
|---------|--|----|
| 2.2 | Chemical Structure and Nature of Rapamycin | 14 |
| 2.3 | Immunosuppressive action of rapamycin | 16 |
| 2.4 | Producer microorganism | 17 |
| 2.4.1 | Biosynthetic gene cluster of <i>S.hygroscopicus</i> for production of rapamycin | 17 |
| 2.4.2 | Precursors for rapamycin biosynthesis | 18 |
| 2.4.3 | Overall biosynthetic pathway of rapamycin | 19 |
| 2.5 | Production of rapamycin | 21 |
| 2.5.1 | Chemical production of rapamycin | 21 |
| 2.5.2 | Microbial production of rapamycin | 21 |
| 2.5.3 | Strain Improvement | 22 |
| 2.5.4 | Optimization of production parameters | 24 |
| 2.5.4.1 | Classical approach | 24 |
| 2.5.4.2 | Statistical optimization | 25 |
| 2.6 | Strategies for modulation of production process | 28 |
| 2.6.1 | Immobilization of producer organism | 28 |
| 2.6.2 | Production in Air Lift Reactor | 29 |
| 2.6.2.1 | Use of airlift reactors for antibiotic production | 32 |
| 2.6.3 | Co-culture of competitor species | 33 |
| 2.6.3.1 | Co-culture for antibiotic production | 34 |
| 2.6.4 | Fed batch strategy | 36 |
| 2.7 | Purification of rapamycin | 39 |
| 2.8 | Stability of rapamycin | 44 |
| | Objectives | 45 |

| | | |
|------------------|--|---------------|
| Chapter 3 | Materials and Methods | 46- 72 |
| 3.1 | Materials | 46 |
| 3.1.1 | Chemicals and Reagents | 46 |
| 3.1.2 | Microorganisms | 46 |
| 3.1.3 | Instruments and Equipments used | 46 |
| 3.1.4 | Media | 48 |
| 3.1.4.1 | Maintenance media | 48 |
| 3.1.4.2 | Seed Culture medium | 49 |
| 3.1.4.3 | Production medium | 49 |
| 3.1.5 | Staining Reagents | 50 |
| 3.1.5.1 | Gram Staining | 50 |
| 3.1.6 | Software used | 50 |
| 3.2 | Methods | 51 |
| 3.2.1 | Maintenance of cultures | 51 |
| 3.2.2 | Visualization under microscope | 51 |
| 3.2.2.1 | Light microscopy | 51 |
| 3.2.2.2 | Scanning Electron Microscopy | 52 |
| 3.2.3 | Production of Rapamycin | 54 |
| 3.2.3.1 | Inoculum Preparation | 54 |
| 3.2.3.2 | Shake Flask Studies | 54 |
| 3.2.3.4 | Production in Stirred tank Bioreactor | 55 |
| 3.2.3.5 | Production of rapamycin in 3L airlift bioreactor | 55 |
| 3.2.4 | Optimization of media components | 59 |
| 3.2.4.1 | Study of interactive effect of parameters with Central Composite Design (CCD) | 59 |

| | | |
|------------------|--|----------------|
| 3.2.4.2 | Artificial neural network (ANN) | 59 |
| 3.2.4.3 | Genetic algorithm | 62 |
| 3.2.5 | Study of immobilization of <i>Streptomyces hygroscopicus</i> | 63 |
| 3.2.5.1 | Measurement of porosity of carriers | 65 |
| 3.2.5.2 | Pre-treatment of carriers | 65 |
| 3.2.5.3 | Cell Immobilization Study | 66 |
| 3.2.5.4 | Cell Release Study | 66 |
| 3.2.6 | Co-culture Technique | 66 |
| 3.2.6.1 | Co-culture on agar media | 67 |
| 3.2.6.2 | Co-culture in liquid media | 67 |
| 3.2.7 | Analytical Techniques | 67 |
| 3.2.7.1 | Estimation of reducing sugar concentration | 67 |
| 3.2.7.2 | Estimation of dry cell mass | 68 |
| 3.2.7.3 | Estimation of rapamycin concentration | 69 |
| 3.2.7.3 | Estimation of k_{La} | 69 |
| 3.2.7.4 | Estimation of broth viscosity | 71 |
| 3.2.7.5 | HPTLC analysis | 72 |
| 3.2.7.6 | Silica column chromatography | 72 |
| 3.2.7.7 | FTIR analysis | 72 |
| Chapter 4 | Results and Discussion | 73- 140 |
| 4.1 | Optimization of medium components using statistical design tools | 73 |
| 4.1.1 | CCD based experimental design | 73 |
| 4.1.1.1 | Regression analysis of the interactions between the media | 75 |

| | | |
|---------|--|-----|
| | components | |
| 4.1.2 | Use of Artificial Intelligence tool for optimization | 77 |
| 4.1.2.1 | Development of ANN based model for rapamycin | 77 |
| | production | |
| 4.1.2.2 | Optimization by genetic algorithm using ANN model | 79 |
| 4.2 | Rapamycin production in 3 L bioreactor | 81 |
| 4.2.1 | Modeling of cell growth, rapamycin production and | 81 |
| | substrate utilization | |
| 4.2.2 | Evaluation of the kinetic parameters | 89 |
| 4.2.2.1 | Evaluation of growth kinetics parameter | 89 |
| 4.2.2.2 | Evaluation of product formation kinetics | 91 |
| 4.2.3 | Dissolved oxygen profile during rapamycin production in | 95 |
| | 3L fermentor | |
| 4.2.4 | Studies on broth rheology in 3L stirred tank reactor | 98 |
| 4.3 | Different strategies for rapamycin production | 106 |
| 4.3.1 | Immobilization of <i>S. hygroscopicus</i> using different carriers | 107 |
| 4.3.1.1 | Growth of <i>S. hygroscopicus</i> on different carriers | 107 |
| 4.3.1.2 | Scanning Electron microscopy of immobilized carriers | 108 |
| 4.3.1.3 | Study of release of cells by immobilized carriers during | 108 |
| | production | |
| 4.3.1.4 | Production of rapamycin using different carriers for | 110 |
| | immobilization | |
| 4.3.1.5 | Production of rapamycin by <i>S. hygroscopicus</i> immobilized | 110 |
| | on PUF for repeated batches | |
| 4.3.2 | Study of rapamycin production in an internal loop airlift | 113 |

| | | |
|---------|--|-----|
| | bioreactor | |
| 4.3.2.1 | Variation of rapamycin production at different aeration rates | 115 |
| 4.3.2.2 | Rapamycin production, biomass generation and substrate depletion | 115 |
| 4.3.2.3 | Determination of kinetic parameters for rapamycin production in ALR | 116 |
| 4.3.2.4 | Estimation of dissolved oxygen concentration for rapamycin production in ALR | 120 |
| 4.3.3 | Study of rapamycin production when <i>S.hygroscopicus</i> was grown with a competitor strain | 122 |
| 4.3.3.1 | Adaptation of <i>S.hygroscopicus</i> for growth in co-culture condition | 122 |
| 4.3.3.2 | Study for optimization of inoculum size of <i>C.albicans</i> | 124 |
| 4.3.3.3 | Study for optimization of inoculation time | 124 |
| 4.3.4 | Fed batch fermentation strategy using pulse feeding | 126 |
| 4.3.4.1 | Study of fed-batch fermentation parameters | 127 |
| 4.3.4.2 | Evaluation of fed batch kinetics | 129 |
| 4.3.4.3 | Study of broth rheology during fed-batch fermentation | 131 |
| 4.4 | Purification of rapamycin | 133 |
| 4.4.1 | Solvent extraction | 133 |
| 4.4.2 | Purification using silica gel column chromatography | 135 |
| 4.4.3 | Qualitative characterization of purified sample using FTIR | 137 |
| 4.4.4 | Quantitative analysis of the purified sample using HPLC | 137 |

| | | |
|------------------|-----------------------------|-----------------|
| Chapter 5 | Conclusion | 141- 145 |
| | Summary and Conclusions | |
| | References | 146- 168 |
| | List of Publications | 169 |
| | Appendix 1 | |
