

STUDIES ON MICROBIAL PRODUCTION OF GIBBERELLIC ACID

by

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CERTIFICATE

This is to certify that the thesis entitled “**Studies on Microbial Production of Gibberellic Acid**” being submitted by **Ruchi Shukla** to the Indian Institute of Technology, Delhi, for the award of degree of Doctor of Philosophy, has been prepared under our supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology, Delhi. The results contained in it have not been submitted in part or full to any other university or Institute for the award of any degree or diploma.



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ABSTRACT

Gibberellic acid (GA₃) is a well-known growth promoting plant hormone with applications in agriculture, horticulture and brewing industry. Growth of *Gibberella fujikuroi* and production of gibberellic acid were studied in different media. Maximum biomass concentration (8.11 g/L) and GA₃ concentration (332.5 mg/L) was obtained in media containing: Glucose – 100 g/L, NH₄Cl – 1.0 g/L, KH₂PO₄ – 3.0 g/L, MgSO₄ – 1.5 g/L, Rice flour – 2.0 g/L. Glucose and ammonium nitrate were found to be most suitable carbon and nitrogen sources respectively, for the growth of *Gibberella fujikuroi* and GA₃ production. The effect of initial C/N ratio on maximum specific growth rate ($\mu_{\max}$) was studied in the range 7.97 – 796.8. Increasing carbon supply and therefore increasing C/N ratio was found to both limit and inhibit the growth of culture. Maximum specific growth rate (0.096 h⁻¹) was observed at C/N ratio of 119.5 and no growth ($\mu = 0$) was observed at C/N ratio of 917.8.

The kinetics of gibberellic acid (GA₃) production and growth of *Gibberella fujikuroi* was studied in a batch cultivation process at pH 5.0 and temperature 30⁰C in 3 L fermenter. It featured 12 g/L accumulation of biomass in the initial 50 h of fermentation followed by GA₃ production of 1 g/L during 50 h to 170 h. The concentration of other metabolites such as lipid and extracellular polysaccharide were found to be 3.1 g/L and 25.05 g/L respectively at the end of fermentation. An unstructured mathematical model was proposed to describe the batch kinetics of fermentation. The model parameters were evaluated using the actual batch cultivation data. The proposed model described well the observed experimental kinetics with respect to substrate consumption, biomass and product formation. The statistical validity of the identified model was demonstrated with

an accuracy of 95 % using 'F' test. The batch kinetic model was extrapolated to computer simulate and design (offline on computer) the nutrient feeding strategies of fed-batch cultivation for production of high concentration and increased productivity of GA₃. Three nutrient feeding strategies for fed-batch fermentations were selected and implemented experimentally. Highest GA₃ concentration (1.82 g/L) and volumetric productivity (18.2 mg/L.h) of GA₃ was obtained by the constant feeding of both glucose (350 g/L) and ammonium nitrate (18.6 g/L) at the rate of 4 mL/h in growth phase (10 h to 40 h) and subsequently feeding of only glucose (300 g/L) at the rate of 4 mL/h in production phase (40 h to 100 h). In multiple fed-batch fermentation, the average volumetric productivity of GA₃ per cycle was found to be 6.2 mg/L.h as opposed to 5.8 mg/L.h in batch cultivation under optimized culture conditions.

After the fermentation, the GA₃ was isolated / concentrated from fermentation broth using polyethyleneimine (PEI) and hydroxyethylcellulose (HEC) based two-phase system. The influence of system parameters (e.g. pH, PEI concentration and phosphate ion concentration) on the isolation of GA₃ was also studied. Under optimum conditions of two-phase system, the GA₃ from fermentation broth was concentrated 2 folds in a single stage with 87.9 % recovery. The gibberellic acid produced in the present investigation was found to be biologically active as confirmed by the enhancement of α -amylase activity in barley endosperm.

CONTENTS

Title	Page No.
List of Figures	i - ii
List of Tables	iii - iv
List of Abbreviations	v
List of Symbols	vi - vii
 CHAPTER 1. INTRODUCTION AND OBJECTIVES	 1 – 6
1.1 Introduction	1
1.2 Objectives	4
 CHAPTER 2. LITERATURE RIVIEW	 7 – 38
2.1 Historical perspectives	7
2.2 Chemical, physical and biological properties of gibberellins	8
2.3 Gibberellic acid producing microorganisms	9
2.4 Biosynthetic pathway of gibberellins (GAs)	11
2.5 Bioprocessing strategies	16
2.5.1 Submerged fermentation	16
2.5.2 Solid-state fermentation	22
2.5.3 Immobilized cell fermentation	25
2.6 Recovery / purification processes	27
2.6.1 Recovery of GA ₃ by aqueous two-phase system	29
2.7 Problems associated with gibberellic acid fermentation	32
2.8 Bioprocess strategies to eliminate above limitations	35
2.8.1 Fed - batch process	35
2.8.2 Continuous culture with cell recycle / cell retention	36
2.8.3 Extractive fermentation process	36
2.8.4 Two-stage process	37

CHAPTER 3. MATERIALS AND METHODS	39 - 55
3.1 Materials	39
3.1.1 Chemicals	39
3.1.2 Equipment	41
3.1.3 Microorganism	41
3.2 Experimental methods	41
3.2.1 Inoculum preparation	41
3.2.2 Effect of different culture media on growth and gibberellic acid production	42
3.2.3 Effect of nitrogen source	42
3.2.4 Effect of carbon source	44
3.2.5 Effect of metal ions	44
3.2.6 Batch cultivation in shake flask	44
3.2.7 Effect of glucose concentration	45
3.2.8 Shake flask inhibition studies	45
3.2.9 Batch cultivation in 3 L fermenter	46
3.2.10 Development of the mathematical model	46
3.2.11 Fed-batch cultivation	47
3.2.12 Multiple fed-batch cultivation	48
3.2.13 Isolation / concentration of GA ₃ from fermentation broth using polyelectrolyte based aqueous two-phase system	49
3.2.13.1 Construction of binodial	49
3.2.13.2 Partitioning of gibberellic acid	49
3.2.13.3 Effect of phosphate ions on the partitioning of GA ₃ in PEI / HEC based ATPS	50
3.2.13.4 Phase diagram of two-phase system consisting of PEI and HEC with fermentation broth as the bulk medium	50
3.2.13.5 Factors affecting the partitioning of GA ₃ in PEI and HEC based ATPS	51

3.3 Analytical methods	52
3.3.1 Dry cell weight (DCW)	52
3.3.2 Estimation of glucose and total nitrogen	52
3.3.3 Estimation of gibberellic acid	52
3.3.4 Estimation of bikaverin	53
3.3.5 Estimation of lipid	53
3.3.6 Estimation of extracellular polysaccharide	53
3.3.7 Estimation of total gibberellins	53
3.3.8 Estimation of total acids	54
3.3.9 α – Amylase bioassay for gibberellic acid	54
3.3.9.1 Assay of α – amylase activity	54
 CHAPTER 4. RESULTS AND DISCUSSION	 56 – 109
4.1 Media screening	56
4.2 α – Amylase bioassay for gibberellic acid	58
4.3 Effect of nitrogen source	59
4.4 Effect of carbon source	60
4.5 Effect of metal ions	62
4.6 Batch cultivation in shake flask	63
4.6.1 Carbon balance	66
4.7 Effect of glucose concentration	67
4.8 Effect of C/N ratio on growth of <i>G. fujikuroi</i>	69
4.9 Batch cultivation in 3 L fermenter under uncontrolled pH	69
4.10 Batch cultivation in 3 L fermenter under controlled pH	73
4.11 Model development for batch cultivation of <i>G. fujikuroi</i>	76
4.11.1 Model equations	76
4.11.2 Statistical validity of the mathematical model	84
4.12 Mathematical model for fed-batch cultivation	85
4.12.1 Fed-batch cultivation I	87
4.12.2 Fed-batch cultivation II	89
4.12.3 Fed-batch cultivation III	90

4.13 Multiple fed-batch cultivation	92
4.14 Comparison of parameters for growth of <i>G. fujikuroi</i> and product formation in batch and fed-batch cultivations	95
4.15 Product isolation / concentration by aqueous two-phase system	97
4.15.1 Phase diagram	97
4.15.2 Partitioning of GA ₃ in PEI / PEG, PEI / HEC, PEI / PVD and PEI / PVA aqueous two-phase system	99
4.15.3 Effect of phosphate ions on the partitioning of GA ₃ in PEI / HEC based ATPS	100
4.15.4 Isolation / concentration of GA ₃ from fermentation broth by polyelectrolyte based (PEI / HEC) ATPS	101
4.15.4.1 Phase diagram of two-phase system consisting of PEI and HEC in the presence of fermentation broth	101
4.15.4.2 Factors affecting the partitioning of GA ₃ in PEI and HEC based ATPS	103
 CHAPTER 5. CONCLUSION	 110 – 112
 REFERENCES	 113 – 135
 APPENDICES	