# STUDIES ON MICROBIAL PRODUCTION OF GIBBERELLIC ACID

by


**RUCHI SHUKLA**

**DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY**


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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.

**Prof. Subhash Chand**
Professor
Department of Biochemical Engineering
& Biotechnology,
Indian Institute of Technology, Delhi
New Delhi – 110016
India

**Prof. A.K. Srivastava**
Professor
Department of Biochemical Engineering
& Biotechnology,
Indian Institute of Technology, Delhi
New Delhi – 110016
India

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# ABSTRACT

Gibberellic acid ($GA_3$) 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 $GA_3$ concentration (332.5 mg/L) was obtained in media containing: Glucose – 100 g/L, $NH_4Cl$ – 1.0 g/L, $KH_2PO_4$ – 3.0 g/L, $MgSO_4$ – 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 $GA_3$ 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 ($GA_3$) production and growth of *Gibberella fujikuroi* was studied in a batch cultivation process at pH 5.0 and temperature $30^0$C in 3 L fermenter. It featured 12 g/L accumulation of biomass in the initial 50 h of fermentation followed by $GA_3$ 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 $GA_3$. Three nutrient feeding strategies for fed-batch fermentations were selected and implemented experimentally. Highest $GA_3$ concentration (1.82 g/L) and volumetric productivity (18.2 mg/L.h) of $GA_3$ 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 $GA_3$ 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 $GA_3$ 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 $GA_3$ was also studied. Under optimum conditions of two-phase system, the $GA_3$ 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 $\alpha$-amylase activity in barley endosperm.

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