ABSTRACT

The increased awareness and application of biological pesticides in agricultural sector has motivated the research towards its economical production. The crystalline protein named insecticidal crystal protein (ICP) synthesised at the onset of sporulation by *Bacillus thuringiensis* is lethal against specific pests. Attempts were made to enhance the synthesis of biomass and ICP by *Bacillus thuringiensis* var *galleriae* (*Btg*) employing process optimization strategies. The process optimization was carried out with heat flux calorimetry in pilot scale bioreactor and glucose concentration control in bench scale bioreactor.

The 300 l pilot scale bioreactor was modified for heat measurements during the cultivation. Modifications include installation of temperature probes and flowmeter in cooling water jacket circuit, wattmeter for measuring the power consumed by stirrer and calibration heater. Data acquisition program for the online monitoring of different fermentation variables and cascade control system for the maintenance of bioreactor temperature were developed in visual programming environment, LabVIEW. These online monitored variables were used in the estimation of individual heat transfer fluxes during cultivation. The heat flux model for overall heat transfer rate in pilot scale bioreactor was applied for the determination of microbial metabolic heat production rate.
Successful demonstration of the feasibility in online monitoring and quantification of metabolic heat flux on cultivation was carried out. Further, fedbatch cultivations in complex medium were carried out with feeding concentrated glucose solution in proportions to the metabolic heat production rate. It was identified with feeding glucose solution at 4X of 26 g/l glucose on the basis of heat signal to yield ICP at a high concentration of 6.969 g/l. The significance of complex sources as nutrients towards the synthesis of ICP was studied. In addition, it was observed that the temperature profile of water in the jacket could be used as an online monitoring tool for continuous assessment of metabolic status of the culture during cultivation.

In the second part of the work, biochemistry analyzer was configured for online monitoring of residual glucose concentration in the bioreactor. A fuzzy logic based feedback control system for maintaining the residual glucose concentration at a constant desired level during cultivation from its online measured concentration was developed in LabVIEW. This control system indicated the possibility in providing a balanced substrate flux during cultivation. Initially, batch cultivations were performed with varying initial concentration of glucose as carbon source and yeast extract as nitrogen source to identify the optimum residual glucose concentration favoring balanced flux of nutrients towards biomass and ICP synthesis. This identification was carried out on the basis of instantaneous growth rate and instantaneous substrate utilization rate from the simulated data obtained from batch cultivations. The observed optimum level of 2.72 g/l in residual glucose concentration was
maintained by fedbatch cultivation with feeding glucose and yeast extract at equal concentration ratio with the above control system. High cell density of 15.992 g/l with specific growth rate of 0.688 was obtained during the exponential growth phase. The balanced flux of substrate during cultivation has resulted in the enhanced synthesis of biomass and ICP. This optimized process could be commercially exploited by comparing the fluxes of basal compounds in different media sources used in fermentation.