Microbial heat produced during the fermentation of *B.t.g* was well correlated with other on-line process parameters such as oxygen uptake, carbon dioxide production and dissolved oxygen concentration. Heat measurement combining with mass and energy balances enabled to estimate absolute yield coefficients with media containing complex nutrients. Thus calorimeter has shown promising application for biopesticide production including its use as on-line process monitoring tool, redundancy check on process variables and calculating for accurate stoichiometry of the growth reaction.

Amino acid analysis using OPA pre-derivatization followed by separation on reversed-phase column was successfully implemented in estimating the utilisation of amino acids during fermentation process with media containing complex proteins. Crystals were separated from spore-crystal suspension using density gradient centrifugation method. The crystals were then separated on SDS-PAGE and estimated for the active endotoxin content using image analysis. A sensitive and reliable ELISA based quantification method was developed to estimate the insecticidal crystal protein produced from different batch experiments using different media.

Amino acids were identified as growth limiting nutrients during the batch cultivation of *B.t.g* using GY medium. GYA medium containing set of nine amino acids influenced both biomass and toxin production followed by increase in glucose uptake. Increasing concentrations of these set of nine amino acids in GYA medium inhibited the crystal protein production. However, GYAA medium enhanced biomass and endotoxin
production further, revealing the need for the addition of yeast extract comprised other amino acids apart from the amino acids of GYA medium for crystal protein production. The biomass concentration was increased from 1.18 g/l to 3.06 g/l using GYAA medium from GY medium. Also, there was a 3-fold increase in endotoxin produced per gram cell weight from using GY to GYAA medium. Glucose depletion by the end of the growth phase was found to be prerequisite for a predominant crystal formation. Batch cultivation using high concentrations of glucose and amino acids did not show any inhibitory effect either on biomass or crystal protein production. Systematic studies using high concentrations of glucose and amino acids in batch cultivation gave a maximum biomass concentration of 11.5 g/l and 6.5 times more endotoxin per gram cell weight from using GY medium.

These studies on amino acids supplementation and glucose uptake could be used further in biopesticide production
(i) For the design of optimal feed profiles in Fed-batch processes
(ii) In the design of complex media for industrial processes containing the required amounts of amino acids.