INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	ASHISH A PRABHU
Roll Number	:	136106019
Programme of Study	:	Ph.D.

Thesis Title: PROCESS DEVELOPMENT FOR THE PRODUCTION OF RECOMBINANT HUMAN INTERFERON GAMMA (hIFN-γ) in *Pichia pastoris* CELL FACTORY

Name of Thesis Supervisor(s)	:	PROF VENKATADASU VEERANKI
Thesis Submitted to the Department/ Center	:	BIOSCIENCES AND BIOENGINEERING
Date of completion of Thesis Viva-Voce Exam	:	09/03/2018
Key words for description of Thesis Work	:	*Pichia pastoris*, human interferon gamma, metabolic engineering, process optimization, protein purification

SHORT ABSTRACT

Human interferon gamma (hIFN-γ) is a pleiotropic cytokine that is produced by natural killer cells and T lymphocytes. hIFN-γ plays an key role in communicating innate and acquired immune systems during bacterial and viral infections, it also possess antiviral, immunoregulatory, and anti-tumor properties. In the present study, cell level and process level strategies were applied to address the expression machinery related and process related problems, which are bottle neck for protein expression in *Pichia pastoris*. Co-expression of chaperones and codon optimization enhanced the production of hIFN-γ to 2.5 mg/L. Further medium development for high level expression of hIFN- γ from *Pichia pastoris* (GS115) was performed with the aid of statistical and nonlinear modeling techniques. Sequential optimization of modified FM22 medium with RSM and ANN-GA resulted in 30 mg/L of hIFN-γ production. The validation was carried out in batch bioreactor and unstructured kinetic models were adapted. The Luedeking-Piret (L-P) model showed production of hIFN-γ was mixed growth associated with the maximum production rate of 40 mg/L of hIFN-γ production. . Different substrate inhibition models were fitted to the growth kinetic data and the additive form of double webb model was found to be the best to explain the growth kinetics of recombinant *P.pastoris*. A novel purification strategy for recombinant human interferon gamma (rhIFN-γ) using nickel chelated metal affinity reverse micellar extraction was demonstrated. The development of this purification system with optimized parameters led to an efficient recovery of 67.3% and improved purity of 79.54%. The anti-proliferative activity on A431 cell lines showed that 50 % inhibition with 80 ng/ml rhIFN-γ concentration and the cells showed necrotic activity. Pathway engineering by overexpressing oxidative enzymes in Pentose Phosphate Pathway (PPP) was carried out to

enhance the expression level of hIFN-γ. synergetic effect of 6-Phosphogluconolactonase (SOL3) and D-Ribulose-5-phosphate 3-epimerase (RPE1) resulted in 2.56 fold increase in hIFN-γ compared to control. The fed batch studies with gluconate/methanol as carbon source enhanced the hIFN-γ to 80 mg/L and 123 mg/L in *Pichia* GS115/hIFN-γ and GS115/hIFN-γ/SR respectively. To get more insight of the flux distribution towards hIFN-γ, studies were carried out by applying flux balance analysis during methanol fed batch phase for both strains. In both strains (GS115/hIFN-γ and GS115/hIFN-γ/SR) more than 95% of formaldehyde flux is directed towards assimilatory pathway. The analysis revealed that with overexpression of SOL3 and RPE1 the flux towards PPP triggering the alleviation in hIFN-γ production.