INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Process Analytical Technology (PAT) Control Tools for High Cell Density Cultivation of Glycoengineered *Pichia pastoris* for Human Interferon α2b Production

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SHORT ABSTRACT

Interferons are a group of multifunctional secreted proteins categorized as cytokines involved in intracellular signalling. Human interferon α2b (huIFN α2b) is a type I interferon and one of 13 variants of interferon α that have been reported. huIFN α2b triggers many biological activities such as antiviral, antiproliferative, and immunomodulatory functions. As a result, it is used in the treatment of hepatitis B and C, hairy cell leukaemia, melanoma, and AIDS-related Kaposi's sarcoma. In the context of recombinant protein production, yeast expression systems, particularly *Pichia pastoris*, offer advantages over bacterial hosts. They facilitate the synthesis of glycosylated recombinant proteins through appropriate post-translational modifications and entail lower operational costs than mammalian cell lines. However, challenges arise in managing higher expression, leading to the accumulation of improperly folded proteins in the Endoplasmic Reticulum (ER). Inappropriately/unfolded protein fractions in culture supernatants require extensive purification steps and are strongly discouraged from the Quality by Design (QbD) perspective. In accordance with the Process Analytical Technology (PAT) initiative, the critical process parameters (CPPs) and critical quality attributes (CQAs) of the process need to be identified and monitored in real-time to achieve improved product quality. Therefore, optimization of the protein titer from *P. pastoris* could be achieved by developing relationships between the various CPPs/CQAs and product titers. Cultivation of *P. pastoris* by manipulating the specific growth rate (μ), a CPP, has a stronger influence at the metabolic level. Therefore, controlling μ in real-time leads to enhanced product output. However, deconvolution of the soft sensor/online sensor input into CPPs is at the nascent stage for most therapeutic protein production.

The first objective of the thesis focussed on appropriate selection of metabolic heat rate based soft sensors for the real-time estimation of μ. Accurate and reliable estimation of μ in real-time is pivotal for reliable monitoring of bioprocesses and the subsequent implementation of advanced control strategies. However, the rationale behind selecting a suitable estimator model from a calorimetric perspective remained unexplored. the notion behind the selection of an appropriate estimator for μ, and the assessment of the estimator models was illustrated using different types of energy metabolism. The second objective focused on the robust control of μ in glycoengineered *P. pastoris* for the production of huIFN α2b. Reliable real-time μ values from the cumulative heat-based soft sensor

were considered as input values for the control strategies employed. The third objective dealt with the effect of methanol concentration on huIFN α2b production. Similar to any other recombinant protein produced in the methylotrophic yeast *P. pastoris,* huIFN α2b production in *P. pastoris* depends on methanol utilization during the induction phase. The impact of varying residual methanol concentrations on huIFN α2b productivity and assessed control strategies for the tight control of residual methanol concentration. Final objective focussed on combinatorial approach of controlling μ through optimal feeding of methanol during the induction phase Since μ and residual methanol concentration influenced huIFN α2b productivity. Model predictive control (MPC) has emerged as a robust approach to realise enhanced control over process parameters in bioprocesses.

In summary, the approach presented in this thesis follows the measuring, modelling, monitoring, and control (M3C) strategy. The integration of the M3C strategy in bioprocess development will impart quality to the process and significantly improve product quantity and quality.