**INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI**
**SHORT ABSTRACT OF THESIS**

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Thesis Title:
Advanced process control for continuous bioprocessing of biotherapeutic protein production

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## SHORT ABSTRACT

The global biotherapeutics market has been tremendously growing with a compound annual growth rate (CAGR) of 13.8%, and is expected to reach a total sales value close to half a trillion USD within the next five years. The rising demand for the production of high-quality therapeutic products in affordable price range, especially in developing countries, has made the manufacturers to focus on the implementation of a continuous manufacturing process for the production of the same. Efficient measurement and control of the critical process parameters (CPPs) governing the critical quality attributes (CQAs) of the therapeutic products can be achieved by implementing the mechanisms of Process analytical technology (PAT) as imparted by the FDA. The work presented herein attempts to implement the different components of PAT to venture the possibility of acquiring reliable real-time process measurements and utilizing them in a process model to develop an efficient control strategy. The expression of the therapeutic product Ranibizumab (also called Lucentis) in recombinant *Escherichia coli* has been chosen as the system for exploring the various concepts of PAT.

The physiological changes associated with cellular population would impact product quality, and therefore, real-time monitoring and estimation of the biomass concentration using an emerging PAT tool, namely, dielectric spectroscopy (DS), was explored in the first phase of this study. The real-time scanning capacitance data from DS were pre-processed and then modelled through a nonlinear theoretical Cole-Cole model. The obtained model parameters were further applied to estimate the physiological properties like cell diameter and viable cell concentration (VCC), which were validated using traditional offline analytical methods like particle size analyzer and flow cytometry, respectively. The Cole-Cole model predicted the cell diameter and viable cell concentration with an error of 1.03% and 7.72%, respectively. The proposed approach can enable the operator to take real-time process decisions to achieve desired productivity and product quality.

The second phase of the study focused on developing a mechanistic model based on mass balances of the various state variables of fermentation and the application of the model to optimize the total biomass with the aid of online capacitance measurements. The developed mechanistic model was validated using experimental data sets obtained from the production of a therapeutic product, Ranibizumab, from *E. coli*. The model predicted the experimental results of the calibration set and validation set within an average error value of 12.64% and 14.97%, respectively.

The final phase of the study focuses on the development of different optimization case studies for achieving enhanced productivity. The objective of the case study (1) focused on maximizing the total biomass in the reactor at a minimum broth volume. A validated mechanistic model was employed to formulate a multiobjective optimization (MOO) problem. The substrate flow rate during the fed-batch phase ($F$) was taken as the decision variable for the MOO. The Pareto front resulting from MOO revealed that for a minimum broth volume ($V$) of 1.96 L, a maximum of 58.8 g of total biomass ($XV$) could be generated. The total biomass obtained from the optimal substrate feeding profile was 20.6% higher than the experimentally achieved total biomass. Enhanced productivity was achieved by the proposed MOO formulation, which facilitates the choice of any operating point from the Pareto front based on downstream expenses of the therapeutic product.

The case study (2) focuses on the development of optimization strategies for predicting an optimal fed-batch harvest time. The harvest of a batch is typically linked to the time of induction. Rather than using time as the control criterion, basing harvest on biomass concentration is likely to result in more consistent process performance. The previously developed MOO was used along with a third objective of optimizing final harvest time $t_f$ ($t_{end}$). Simulation studies were carried out with different $t_{end}$ values to predict the optimal fed-batch harvest time. The Pareto for different $t_{end}$ values were obtained, and the objective functions were compared at different $\lambda$ values. The optimal feeding profiles and fed-batch harvest time can be chosen based on the desired volume of operation.

In a nutshell, the approach presented in this work integrated the real-time process measurements in a validated process model and explored the application of different optimization strategies for a therapeutic protein production process. The combination of enhanced measurement, modelling and control strategies will significantly improve the product quantity and quality, thereby paving way for better process performance.