USING EXPERIMENTAL AND THEORETICAL APPROACHES FOR UNDERSTANDING BIOTECHNOLOGY UNIT OPERATIONS

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OCTOBER 2017

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by

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Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the

Department of Chemical Engineering

INDIAN INSTITUTE OF TECHNOLOGY DELHI

October 2017

Certificate

This is to certify that the thesis entitled **"Using Experimental and Theoretical Approaches for Understanding Biotech Unit Operations "** being submitted by **Sandeep Ramesh Hadpe** to the Indian Institute of Technology, Delhi, for the award of the degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by him. **Sandeep Ramesh Hadpe** has worked under my guidance and supervision and has fulfilled the requirements for the submission of the thesis.

The results contained in this thesis have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma.

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Acknowledgements

I would like to express my special appreciation and thanks to my advisor Prof. Anurag Rathore, who has been a tremendous mentor for me. I would like to thank him for encouraging my research and for providing me sufficient time to ensure that I complete my research work while taking my job responsibilities. I will always remain indebted to you for your kind words not only on academic related but also on personal front so that I remained focused on my work. The advice given by my SRC members, Prof. Ratan Mohan, Prof. Munawar sheikh and Prof. Tapan Choudhury has been a great help in maintaining the quality of my research work. Their consistent review of my work motivated me to strive harder.

It would not be possible to list down all the people who supported me in the various ways during my research period. However, I would like to thank each and all of you for your direct and indirect contribution to this work. First of all, I would like to mention special thanks to Syngene Vice President, Dr. Dhananjay Patankar; my manager and mentor Solomon Alva; my group lead and industrial supervisor Dr. Jyoti Iyer, Biocon vice president Dr. Anuj Goel and Biocon ex CEO, Dr. Harish Iyer, who steered my thought process to pursue PhD in Bioprocess and also played key role in directly or indirectly supporting me in my decision of pursuing PhD while working in industry.

I am very fortunate to be blessed with friends like Rahul, Rakesh, Neeraj, Suhas,Yogesh and Amit with whom I share all my personal and professional issues; and who went out of their way to help me out whenever needed. Rahul, Rakesh, Suhas and Neeraj also helped me with frequent discussions, which gave me insights on various issues related to my research.

My work would not have been successful or nearly as fun without the support of my colleagues at work, the ever inspiring MDL team Arindam, Anjali, Vinay, Vikram, Himangi, Savi, Meleena, Siddhartha, Hari, Vijay S., Anindita, Ashank, Rajkumar, Satish, Deepak, Manju; colleagues from other departments; Sekhar, Vel, Charan, Gangadharam, Pratap bade, Ganesh T., Vivek Ferkade,

Sagar and colleagues at IIT Delhi research lab; Vijesh, Vishwanath, Nikhil, Sumit, Viki, Milli, Nidhi, Varsha for providing cooperation, support and pleasant research environment to carry out my work smoothly. Also, a special thanks to our ADC group (Abhilashi, Yogesh, Suhas, Neeraj, Saurabh, Somu, Ganna and Babbu) for engaging me in all the inspiring and encouraging discussions to make research fun at work. I would like to extend my special gratitude to Vipin, Harshit, Abhishek and Vijay Maranholkar for their support and help whenever required in my research work.

I am deeply indebted to my family, specially Mom, Dad, Bro (Rahul) and Didi (Varsha), who gave unconditional support to my ambitions and without which I would not have made it so far in my career without the drive, dedication, strength and perseverance that you have instilled in me. Words cannot express how grateful I am to the little chams of my family, Son (Sanky), Nieces (Sakshi & Ishu), Nephews (Krishna & Shreeraj) for providing me the stressbuster moments whenever I needed. Finally, I dedicate this work to my wife, Pallavi, who accepted me along with my commitment towards PhD research. I thank her for her patience, sacrifice, support and understanding during the PhD tenure. All of these members' prayers were instrumental in me being able to sustain this long and tough journey.

Sandeep

Abstract

Improvements in expression technology and cell culture processing accompanied by the increasing volume of bioreactors have resulted in harvests with a high antibody titre and have created the bottleneck in the downstream processes of monoclonal antibody therapeutics. Depth filtration alone or in combination with centrifugation and Protein A chromatography dominate the clarification and capture options respectively. The outcome of upstream improvement forced these two operations to face challenge with respect to capacity, robustness, cost as well as impurity clearance. The commonly known issues with depth filtration include limitations in handling higher solid contents, higher operating cost due to exorbitantly oversized filtration area and requirement of higher flushing. On the other hand, protein A chromatography faces issues including higher adsorbent cost, caustic incompatibility, low pH antibody stability and protein A ligand leaching. The work detailed in this thesis attempts to employ tools like empirical /mechanistic based models or alternate scouting approach to address some of the above mentioned issues.

The first two chapters of this thesis include introduction to the topic and a detailed literature review respectively. The third chapter focuses on development and application of a novel ANN model based approach for predicting depth filter capacity with the help of an industrial case study and addressing concerns related to operating cost of depth filtration due to the significant oversizing of filter area that is practiced in the industry. The fourth chapter presents a comparison of different feed pretreatment alternatives, and their impact on process contaminant removal and filter sizing improvement for depth filtration. This chapter also compares key response parameters of different depth filtration based approaches used for clarification. The fifth chapter evaluates the performance of an Alternating Tangential Flow (ATF) operation. In this relatively new technology, inherent back flushing occurs and has been demonstrated to be superior to the traditional TFF operations (microfiltration/ultrafiltration) in handling fouling feeds like high cell density cell culture stream. The work mentioned in this chapter attempts to develop and compare

mechanistic models for predicting fouling mechanism of ATF and TFF for the use of antibody cell culture clarification. The sixth chapter focuses on scouting of protein A adsorbent for antibody capture. In this chapter, fourteen commercially available protein A candidates have been compared using a Multi Criteria Decision Making (MCDM) tool for it's ranking and selection. Finally, the seventh (last) chapter concludes the research work with detailing the future scope of research in this area.

सार

बायोरेक्टर्स की बढ़ती हुई मात्रा के साथ अभिव्यक्ति प्रौद्योगिकी और सेल संस्कृति प्रसंस्करण में सुधार के कारण उच्च एंटीबॉडी टाइट्रे के साथ फसल हुई है और मोनोक्लोनल एंटीबॉडी चिकित्सीय के डाउनस्ट्रीम प्रोसेसिंग में बाधा उत्पन्न की है। गहराई निस्पंदन अकेले या केन्द्रापसारक और प्रोटीन के साथ संयोजन में एक क्रोमैटोग्राफी क्रमशः स्पष्टीकरण और कब्जा विकल्प पर हावी है। अपस्ट्रीम सुधार के परिणाम ने क्षमता, मजबूती, लागत और अशुद्धता निकासी के संबंध में चुनौती का सामना करने के लिए इन दो आपरेशनों को चुनौती दी। गहराई से निस्पंदन के साथ सामान्यतः ज्ञात मुद्दे में उच्च ठोस सामग्री को संभालने में सीमाएं शामिल हैं, अत्यधिक परिचालन क्षेत्र के कारण उच्च परिचालन लागत और उच्च फ्लशिंग की आवश्यकता। दूसरी ओर, प्रोटीन ए क्रोमैटोग्राफ़ी में उच्च adsorbent लागत, कास्टिक असंगति, कम पीएच एंटीबॉडी स्थिरता और प्रोटीन ए लिगैंड लेचन सहित मुद्दों का सामना करना पड़ता है। इस थीसिस में विस्तृत काम, उपर्युक्त मुद्दों में से कुछ को संबोधित करने के लिए प्रजनन / यंत्रवत आधारित मॉडल या वैकल्पिक स्काउटिंग दृष्टिकोण जैसे उपकरणों को नियोजित करने का प्रयास करता है।

इस थीसिस के पहले दो अध्यायों में विषय के परिचय और क्रमशः विस्तृत साहित्य की समीक्षा शामिल है। तीसरा अध्याय एक औद्योगिक मामले के अध्ययन की सहायता से गहराई से फिल्टर क्षमता की भविष्यवाणी के लिए एक उपन्यास एएनएन मॉडल आधारित दृष्टिकोण के विकास और आवेदन पर ध्यान केंद्रित करता है जिसमें गहराई से छानने के परिचालन लागत से संबंधित चिंताओं को संबोधित किया जाता है, जो कि फिल्टर क्षेत्र के महत्वपूर्ण आधिकरण के कारण होता है। उद्योग। चौथा अध्याय विभिन्न फीड पूर्व-उपचार विकल्पों की तुलना करता है, और प्रक्रिया संदूषण हटाने और गहराई से निस्पंदन के लिए सिज़ाव के सुधार पर उनके प्रभाव को दर्शाता है। यह अध्याय स्पष्टीकरण के लिए उपयोग किए जाने वाले विभिन्न गहराई से निस्पंदन आधारित तरीकों की महत्वपूर्ण प्रतिक्रिया मानकों की तुलना करता है। पांचवें अध्याय एक ऑल्टरनेटिंग टैन्जलिंग फ्लो (एटीएफ) ऑपरेशन के प्रदर्शन का मूल्यांकन करता है। अपेक्षाकृत नई तकनीक में, निहित परत निस्तब्धता होती है और उच्च सेल घनत्व सेल संस्कृति स्ट्रीम जैसे दूषण फीड्स को संभालने में पारंपरिक टीएफएफ संचालन (माइक्रोफिल्टरेशन / अल्ट्राफिल्टरेशन) से बेहतर प्रदर्शन किया गया है। इस अध्याय में वर्णित कार्य एंटिबॉडी सेल संस्कृति स्पष्टीकरण के उपयोग के लिए एटीएफ और टीएफएफ के दूषण तंत्र की भविष्यवाणी के लिए यंत्रवत् मॉडल विकसित और तुलना करने का प्रयास करता है। छठे अध्याय एंटीबॉडी कैप्चर के लिए प्रोटीन एक एसोसोर्न्ट के स्काउटिंग पर केंद्रित है। इस अध्याय में, चौदह व्यावसायिक रूप से उपलब्ध प्रोटीन एक उम्मीदवार की तुलना रैलींग और चयन के लिए एक बहु मानदंड निर्णय लेने (एमसीडीएम) उपकरण से की गई है। अंत में, सातवें (अंतिम) अध्याय इस क्षेत्र में अनुसंधान के भविष्य के दायरे का विवरण देने के साथ शोध कार्य निष्कर्ष निकाला है।

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Nomenclature

Abbreviations	Description
ATF	Alternating tangential Flow
TFF	Tangential flow filtration
TMP	Transmembrane pressure
P1	Feed Pressure
P2	Controller/Retentate Pressure
P3	Permeate Pressure
R	Overall Membrane Resistance
R	Tangential flow rate to permeate flow rate ratio
ΔP	Pressure drop
M	Viscosity of the fluid
L	Thickness of the membrane
Q	Volumetric flow rate
$\mathbf{A_u}$	Unblocked and porous membrane area
$\mathbf{r_p}$	Radius of membrane pore
C	Concentration of foulant
$\mathbf{C_0}$	Initial concentration of the foulant
$\mathbf{V_0}$	Initial harvest volume
$\mathbf{V_P}$	Cumulative permeate volume
$\boldsymbol{\alpha}$	Complete pore blockage parameter
$\mathbf{K_{cpb}}$	Complete pore blockage coefficient
$\boldsymbol{\alpha}$’	Intermediate pore blockage parameter
$\mathbf{K_{ipb}}$	Intermediate pore blockage coefficient
$\mathbf{R_c}$	Cake resistance
$\mathbf{R_m}$	Membrane resistance
A	Area of the membrane
$\mathbf{f'}$	fraction of cells contributing to cake growth
$\mathbf{R'}$	specific cell layer resistance
$\mathbf{K_{cf}}$	cake formation coefficient
$\boldsymbol{\alpha_{in}}$	volume of foulant constricting pore s per unit mass of the potential foulant
$\mathbf{K_{pc}}$	pore constriction coefficient
K	Fouling coefficient
N	Generalized exponent
LHS	Left hand side of equation (33)
CI	Relative closeness to the ideal solution
D	Database matrix
CI	Relative closeness to the ideal solution
D	Database matrix
I	Rows of matrix A, R and W
J	Columns of matrix A, R and W
M	Number of adsorbents evaluated
N	Number of resin attributes
P	Weighted normalized matrix
P^+	Maximum column value in matrix P (positive benchmarking value)
P^-	Minimum column value in matrix P (negative benchmarking value)

‘ProA 1’ - ‘ProA 14’	Protein A adsorbent identification
R	Non-dimensional matrix
SI*	Positive separation measure
SI-	Negative separation measure
W	Weight vector
x_i	i^{th} input of ANN network input
x'_i	Normalized value i^{th} input of ANN network input
x_{min}	Minimum value i^{th} input of ANN network input
x_{max}	Maximum value i^{th} input of ANN network input
y_i	i^{th} value of ANN network output
$f(y_i)$	Sigmoidal transfer function
$y_{i,actual}$	ith target output
$y_{i,predicted}$	ith output of network
y'	Average of the observed experimental output
N	Number of data points used for modelling
K	Normalized filter efficiency
TP	volume throughput (ml/ml),
ή	collection efficiency (%),
C_i	initial concentration of solids (mg/ml)
V_f	Volume of feed filtered (ml),
V_m	Volume of filter media (ml),
V_{sc}	Volume of solids captured (L)
V_s	Volume of solids in the feed (L)