# DA ZHAN. PH.D

479-304-9801
Scientist, Benitec Biopharma
dzhang@benitec.com
Personal Website: https://dazhang.org/
LinkedIn - DaZhang


## EDUCATION

**PHD**, Chemical Engineering; University of Arkansas
Thesis: Understanding Membrane Fouling during Cell Harvesting
Major professor: Dr. Ranil Wickramasinghe

**MS**, Bioengineering; Hebei University of Technology
Thesis: Process Intensification of Fermentation Coupling with Foam Separation
Major professor: Dr. Huijie Zheng

**BS**, Bioengineering; Shenyang University of Chemical Technology

## KEY SKILLS & COMPETENCIES

**GENE THERAPY DEVELOPMENT:** Adeno Associated Virus(AAV) gene therapy, Baculovirus Expression Vector System (BEVS): transfection, plaque purification
**PROCESS DEVELOPMENT:** Tangential flow filtration (TFF), Alternating tangential flow filtration (ATF), foam fractionation, FPLC (GE)
**ASSAY DEVELOPMENT:**
**Protein:** High-performance liquid chromatography (HPLC) (Agilent, CA), Enzyme-linked immunosorbent assay (ELISA), Liquid Chromatography-mass Spectrometry (LC/MS), 2D SDS-PAGE (Cytiva, MA)
**DNA:** qPCR/PCR (Bio-Rad, CA), Reverse transcription (RT)-PCR
**VIRUS CLEARANCE:** Robustness validation for Virus Filtration of mAb and AAV Processes
**CELL CULTURE:** Mammalian cell (adherent, suspension, bioreactor, perfusion), insect cell, bacteria fermentation
**MOLECULAR CLONING:** plasmid construction
**PROGRAMMING:**
MATLAB: solving differential equations and scientific plotting
Python: deep learning model and data analysis
PHP, JavaScript, Linux: LAMP stack web server construction
C++, C#, Java: database-oriented application
Cloud computing (using AWS platform): construction of online database for anonymous files sharing
**SCIENTIFIC SOFTWARE**
SPSS Statistics (IBM, NY)
JMP (SAS institute, NC)
Origin: Data Analysis and Graphing Software (OriginLab Corporation, MA)

## CAREER HISTORY

**SCIENTIST;** Benitec Biopharma**;** Hayward, CA 06/2022 - Present
**Process development:** Establish and test scalable processes for Adeno-Associated viral vector (AAV) for gene therapy applications.
**Virus clearance validation:** Design, review and perform GMP standard virus clearance study to validate the current process which is regulatory required for investigational new drug (IND) submissions and before biologics license application (BLA).
**Assay development:** Design and examine in vitro analytical and cell-based assays to evaluate quality of drug products.
**Technology transfer:** Generate, review and transfer documents; engage with CDMO to transfer processes and assays to manufacture GMP-grade materials for clinical studies and ensure product quality.

**GRADUATE STUDENT CO-OP; Biogen;** Cambridge, MA 06/2021 - 09/2021
**Routine work:** Assembling and mounting of Bioreactor, daily sampling (cell count, osmosis of the culture, pH and pO2, pressure record, metabolites analysis using Cedex Bio HT Analyzer)
**Fouling study:** Backwash the filter, foulant identification using LC/MS
**Method development:** Work with Analytical Development team to improve the fouling identification method (in-solution digestion method)
**Machine learning:** Train the Deep learning model with protein properties to predict the fouling potential

**GRADUATE STUDENT CO-OP; Amgen;** Cambridge, MA 04/2021 - 06/2021
**Routine work:** Assembling and mounting of Bioreactor
**Confidential internal studies**

**Fouling study:** Backwash the filter, foulant identification using LC/MS

**RAPID, AIChE; Student Intern, Online** Summer 2020
Complete training on chemical engineering process intensification
Integration of process intensification in Tangential Flow Filtration (TFF) of cell harvest
Leadership training

**Graduate Assistant; University of Arkansas**, AR 09/2016 - 05/2022
**Main responsibilities**: Research projects (see below), proctored exams, grading, office hours
**Teaching assistant** for the following courses:
CHEG 4332 Lab II, 2021 Spring
CHEG 3333 Reactor, 2020 Spring
CHEG 2113 Introduction to Chemical Engineering, 2019 Fall
CHEG 4423 Process control, 2019 Spring
CHEG 3144, Heat/Mass Transport, 2018 Fall
CHEG 4423 Process control, 2018 Spring
CHEG 4163 Separation, 2017 Fall, lecture on distillation column design, exam review
CHEG 4413 Design I, 2017 Spring

**Engineer**;-**Huanzhong Biotechnology**, China 12/2015 - 06/2016
Plasmid construction, fermentation to produce antigen
Purification of antibodies using ammonium sulfate precipitation
Labeling secondary antibody with enzyme for ELISA assay
Sandwich ELISA assay for antibodies titer determination

## PROFESSIONAL SERVICE

**Journal**
Frontiers in Genetics, reviewer

## PROJECTS / PUBLICATIONS / NATIONAL CONFERENCE PRESENTATIONS

### PROJECTS

- **Understanding Fouling Behavior during Perfusion Operation of Cell Culture Harvesting (MAST # 19-4)**

*Sponsored by:* Amgen, Biogen, MilliporeSigma.
*Main responsibilities:* ATF perfusion perfusion culture, 2D SDS-PAGE, LC/MS, perfusion culture, deep learning model
*Duration:* 2019-2022
Perfusion reactor under alternating tangential flow filtration (ATF) is able to reduce fouling (by periodically reversing the direction of the feed flow into the module), but membrane fouling remains a challenge. Previous studies have indicated that CHO cells, cell debris, host cell proteins (HCPs), and DNA as well as anti-foam can all contribute to membrane fouling, and among those factors, HCPs are the most complex foulants. This project was featured by the identification of HCPs using LC/MS. The proteomics study of HCPs includes backwash of the foulants from ATF filter, precipitation, SDS-PAGE, in-gel digestion, and LC/MS. In addition, fouled membranes were gathered from different biopharmaceutical companies to be compared.
The list of identified HCPs in different operation conditions, companies are trained with a machine learning model to provide more comprehensive details of the dataset, such as the prediction of fouling potential of specific HCP. Linear Discriminant Analysis(LDA) was used to classify those host cell proteins into 3 clusters based on the fouling grade. The analysis confirms that the subcellular locations contribute the most to the classification.
The project also includes setting up and running ATF perfusion at university. The bioreactor vessel and controller station are powered by Sartorius Biostat A plus. The ATF perfusion system is from Repligen.

- **Understanding the Performance of the BioOptimal™ Hollow Fiber Microfilter (MAST # 16-5)**

*Sponsored by:* Asahi Kasei Bioprocess America, Inc.
*Main responsibilities:* TFF, cell culture, modeling, results presentation
*Duration:* 2016-2019
Tangential flow filtration (TFF), typically using 0.2 µm pore size membranes, is one of the filtration methods that is used for bioreactor clarification. Major drawbacks of TFF include membrane fouling and product rejection leading to unacceptably low product recoveries. The BioOptimal™ MF-SL microfilter (Asahi Kasei, Glenview, IL, USA), is a commercially available hollow fiber module that contains a reverse asymmetric membrane. The inside surface has pores up to 40 µm in diameter, while the outside surface has 0.4 µm pores.
The BioOptimal™ MF-SL was systematically evaluated using yeast and CHO as a different feed stream. A mathematics model has been developed to describe the variation of flux and transmembrane pressure drop during filtration using reverse asymmetric membranes. The model can be used to estimate the capacity of the filter for a given feed stream. Compared to the traditional symmetrical membrane, the design of open pores hijacks larger foulant works similarly with filter aid which stabilizes the cake formed by CHO cell. Confocal imaging confirmed our secondary foulant hypothesis.

- **Recovery of polymyxin E using fermentation coupling with foam separation**

*Sponsored by:* Natural Science Foundation of China; Natural Science Foundation of Hebei, China.
*Main responsibilities:* bacterial fermentation, foam fractionation, HPLC
*Duration:* 2012-2015
Foam fractionation utilizes the gas-liquid interface to enrich protein, showing great advantages in many natural biosurfactants fermentation such as nisin, surfactin, and polymyxin E. As well as other separation technologies, foam fractionation can be used as an in-situ product removal technology. In practice, rising bubbles caused by the sparger of the bioreactor were collected, in which the produce was enriched. This project systematically investigated different parameters that affected the enrichment, recovery, and inactivity percentage of polymyxin E using fermentation coupling with foam fractionation. The results indicated pH was the most important factor in foam separation of polymyxin E. Additionally, the adsorption mechanism for polymyxin E and cells was studied. The results indicated polymyxin E was mainly adsorbed on the bubble, while the cells were partly in liquid form and partly adsorbed on undesired protein. Undesired protein can be considered as a stabilizer in foam separation of polymyxin E.
The application of fermentation coupling with foam fractionation can be considered as an alternative strategy of ATF perfusion. While membrane-based perfusion experiences fouling issues caused by high produce titer, foam fractionation can provide more stable and continuous throughput, as the principle here is based on adsorption but not size exclusion.

## DA ZHANG, ADDENDUM 2

### PUBLICATIONS

[1] **Zhang D**, Patel P, Strauss D, Qian X, Wickramasinghe SR. Modeling flux in tangential flow filtration using a reverse asymmetric membrane for Chinese hamster ovary cell clarification. Biotechnology Progress. 2021 May;37(3):e3115.
[2] **Zhang D**, Patel P, Strauss D, Qian X, Ranil Wickramasinghe S. Modeling tangential flow filtration using reverse asymmetric membranes for bioreactor harvesting. Biotechnology Progress. 2021 Jan;37(1):e3084.
[3] **Zhang D**, Dong K, Xu D, Zheng H, Wu Z, Xu X. Process improvement for fermentation coupling with foam separation: a convenient strategy for cell recycle. Asia-Pacific Journal of Chemical Engineering. 2015 May;10(3):466-75.
[4] Sundar, V., **Zhang, D**., Qian, X., Wickramasinghe, S. R., Smelko, J. P., Carbrello, C., ... & Zydney, A. L. (2023). Use of scanning electron microscopy and energy dispersive X‐ray spectroscopy to identify key fouling species during alternating tangential filtration. Biotechnology Progress, e3336.
[5] Namila FN, **Zhang D**, Traylor S, Nguyen T, Singh N, Wickramasinghe R, Qian X. The effects of buffer condition on the fouling behavior of MVM virus filtration of an Fc-fusion protein. Biotechnology and bioengineering. 2019 Oct;116(10):2621-31.
[6] Zheng H, **Zhang D**, Guo K, Dong K, Xu D, Wu Z. Online recovery of nisin during fermentation coupling with foam fractionation. Journal of Food Engineering. 2015 Oct 1;162:25-30.
[7] Cui X, **Zhang D**, Zheng H, Wu Z, Cui S, Dong K. Study on the process of fermentation coupling with foam fractionation and membrane module for nisin production. Asia-Pacific Journal of Chemical Engineering. 2014 Jul;9(4):623-8.

### NATIONAL CONFERENCE PRESENTATIONS

- Understanding Fouling Behavior during Perfusion Operation of Cell Culture Harvesting, online. MAST Center Meetings; October 25, 2021; April 12, 2021, October 27, 2020, April 27, 2020
- Understanding Membrane Fouling during a Combined Tangential-flow and Depth Filtration Process. November 2019, Orlando, FL. AIChE.
- Understanding Membrane Fouling during Cell Harvesting. October 2019, Newark, NJ. MAST Center Meeting.
- Understanding Membrane Fouling during Cell Harvesting. May 2019, Fayetteville, AR. MAST Center Meeting.
- Unrevealing the Plugging Mechanisms during a Combined Tangential-flow and Depth Filtration Process. April 2019, Orlando, FL. ACS BIOT.
- Understanding Membrane Fouling during Cell Harvesting. November 2018, Denver, CO. MAST Center Meeting.
- Understanding Membrane Fouling during Cell Harvesting. April 2018, State College, PA. MAST Center Meeting.
- Understanding Membrane Fouling during Cell Harvesting. October 2017, Newark, NJ. MAST Center Meeting.