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STOCHASTIC SIMULATION MODEL DEVELOPMENT FOR BIOPHARMACEUTICAL PRODUCTION PROCESS RISK ANALYSIS AND STABILITY CONTROL

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ABSTRACT

In this paper we develop a stochastic simulation model for biomanufacturing risk analysis by focusing on the production process from raw materials to finished drug substance. By exploring biotechnology domain knowledge, we model how the properties or attributes of each batch dynamically evolve along the production process. We consider main sources of uncertainty leading to batch-to-batch variation, such as raw material biomass, cell culture, and target protein purification. The proposed simulation model allows us to incorporate the underlying physical chemical interactions and also the no-wait constraint in the purification process. It can be used to facilitate biomanufacturing risk management and guide coherent operational decision making (i.e., production scheduling and quality control) so that the stability of bio-drug quality can be improved while efficiently utilizing the resources and speeding up the time to market.

1 INTRODUCTION

The biomanufacturing industry is growing rapidly and becoming one of the key drivers of personalized medicine and advancement of the life sciences, especially with the introduction of cell and gene therapy products into the market. Different from traditional pharmaceuticals, the production of biopharmaceuticals involves live cells which introduce huge uncertainties at different unit operations. Complexity and challenges have also been brought by the frequent launch of new products, induction of personalized bio-therapy (e.g., CAR-T Cell therapy), and new process technologies, such as continuous manufacturing (Otto et al. 2014). There is an increasing interest in the biopharma industry to develop science- and risk-based methodologies that can integrate the information and data collected from each process unit operation, identify the critical risk factors, and guide the end-to-end production process risk management.

Traditionally, chemical process simulation is used to predict the outcome of a certain procedure in a biomanufacturing process. It mainly builds on analytic models and partial differential equations (PDEs) to simulate the biological and chemical dynamics, e.g., thermodynamic properties (Nfor et al. 2009). Such methodologies are often deterministic and focus on a small part of the biomanufacuring process. Operational research (OR) typically focuses on finding the optimal design, planning, and operational

decisions for complex stochastic systems. Mixed integer linear programming (Leachman et al. 2014; Lakhdar and Papageorgiou 2008) and supply chain management methodologies (Fleischhacker and Zhao 2011) have been presented for planning decisions in biopharmaceutical manufacturing. Simulation models (Saraph 2001; Lim et al. 2004; Kulkarni 2015) are also built for biomanufacturing processes under traditional queueing network theory to analyze resource planning, scheduling, and material consumption costs. Martagan et al. (2016, 2017, 2018) explore physical-chemical characteristics and incorporate biology-induced randomness in either fermentation or chromatography stage, and develop Markov decision models to optimize the corresponding operational policies.

However, state-of-the-art OR methodologies developed for biomanufacturing management have several key limitations. First, the existing approaches often focus on developing general methodologies, and few of them explore the pharmaceutical biotechnology domain knowledge (e.g., the underlying physical mechanics causing the interdependence of raw material quality, production process, and bio-drug properties in safety and efficacy). This potentially limits the OR methodology performance as well as its adoption in the real applications. Second, as far as we know, existing approaches tend to focus on limited parts of the biomanufacturing system and there is no appropriate and reliable end-to-end risk management framework guiding coherent biomanufacturing operational decisions. Third, to the authors' best knowledge, there is no science- and risk-based OR methodology developed to facilitate the development of complex, efficient, and flexible biopharmaceutical production systems, where many personalized bio-therapies requiring different production processes share the same manufacturing resources.

In this paper, we develop a simulation model for the entire biopharmaceutical production process from raw materials to the finished drug substance or active pharmaceutical ingredient (API). In simulation modeling, we explore the underlying physical chemical interactions causing the interdependence of raw material quality, production process, and bio-drug properties in safety and efficacy. For given decision policies (i.e., production scheduling and quality control), the simulation models how the properties and attributes of each batch evolve along the production process. We consider key sources of risk, including raw material biomass variation and various uncertainties introduced from each production step in both upstream cell culture and downstream target protein purification. Based on that, a comprehensive simulation analysis framework is established to study the batch-to-batch variation and provide the insights on how to improve the efficiency and stability of production process. In addition, for personalized bio-therapy, the raw material could be each individual patient's own cells, and the production process also depends on the cell properties. Our simulation model could support flexible and personalized biomanufacturing.

The contributions of our study can be summarized as follows. We develop a stochastic simulation model for the biopharmaceutical production process from raw materials to finished drug substance or API, which can facilitate the end-to-end biomanufacturing risk management. *In the simulation model, we explore the biotechnology domain knowledge as well as queueing network modeling. We model the dynamic and stochastic evolution of each batch property along the production process. The proposed simulation model could guide the development of stable, efficient, and flexible biomanufacturing systems.*

The paper is organized as follows. We provide the problem description and briefly introduce the biopharmaceutical production system in Section 2. Then, we explore the biotechnology domain knowledge and develop the simulation model for the production process in Section 3. In Section 4, we conduct a case study on an antibody biodrug production system. We model the key sources of uncertainty and study the system performance under various operational decision making schemes. We conclude in Section 5.

2 BIOMANUFACTURING PROCESS INTRODUCTION AND PROBLEM DESCRIPTION

In this paper, we focus on the biomanufacturing process from raw materials to finished drug substance or API. The main steps of production process include: (1) pre-culture and expansion, (2) fermentation and harvest, (3) centrifugation(s), (4) chromatography/purification, (5) filtration, and (6) quality control. Steps (1)–(2) belong to upstream cell culture process and Steps (3)–(6) belong to downstream protein purification process. Here, we consider a biomanufacturing system producing various antibody bio-drugs

for illustration. Suppose the production of Type A bio-drugs requires external media. For Type B drugs, we need to prepare the media internally. Once receiving the drug orders, the production process starts based on upstream and downstream releasing policies. The logic of the production process is illustrated in Figure 1. For simplification, suppose that there is always enough inventory of external media. The production procedure for each batch of drug substance mainly includes upstream cell culture process (USP) and downstream target protein purification (DSP).

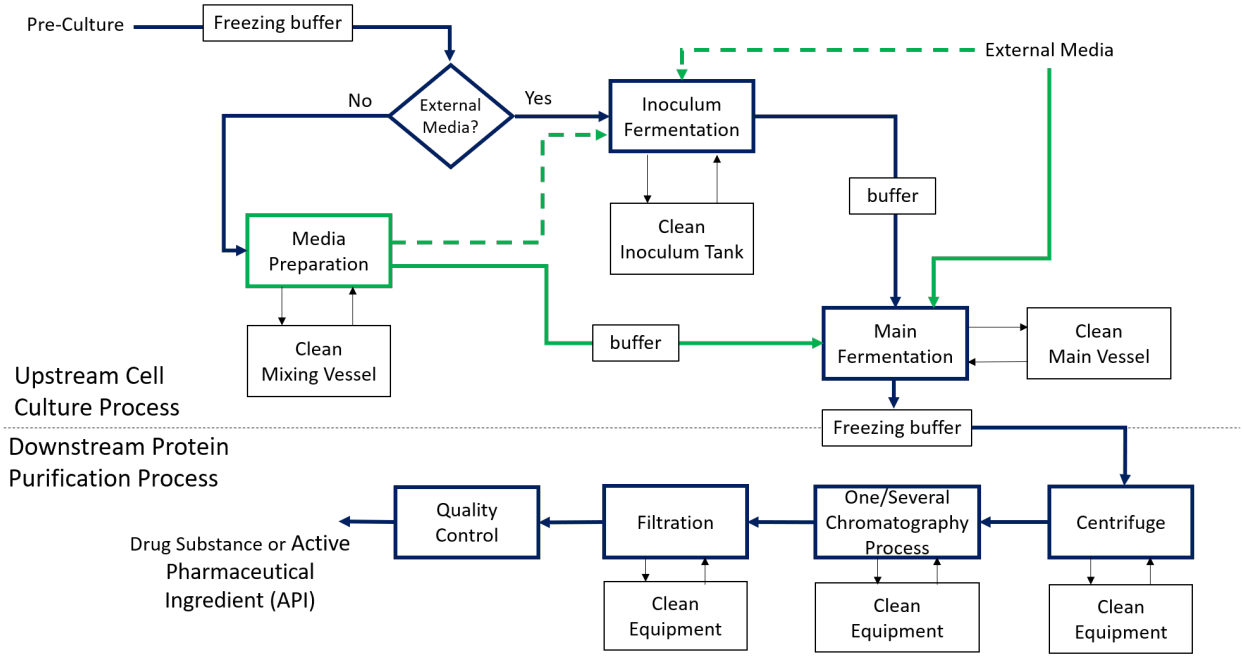

Figure 1: Drug substance production process.

USP starts with pre-culture. Type A bio-drug directly starts inoculum fermentation. Finished inoculum is moved to main fermentation vessel and start main fermentation. For producing any type B drug, the corresponding media must be prepared before it starts the inoculum fermentation and main fermentation. Media is a mixture of nutrients for cell growth or culture. It has a short lifetime (e.g., 2 hours used in the empirical study). The media needs to be discarded and re-prepared if the waiting time exceeds that limit. For simplification, suppose that media used for inoculum and main fermentation are prepared separately. The downstream process is the same for both types of drugs. After the product is released to the downstream, it starts with the centrifuge procedure, followed by one or multiple chromatography steps. Then, the purified product goes through the filtration stage and final quality control before the final product substance of API is obtained. After each step except for quality control, the used equipment needs to be cleaned.

During the production process, the main fermentation is critical and it takes up the biggest portion of the production time. The resulting protein from main fermentation is usually fragile, inquiring the "no-wait" constraint in the following downstream purification process. The no-wait constraint implies that the product cannot wait between different steps. If there is no corresponding resource available during DSP, the protein will degrade and will be discarded. In addition, due to the impurity percentage constraint of the final product, certain "hopeless" batches need to be discarded after the main fermentation stage. This saves the downstream purification resources for batches that could meet the final drug quality requirement and contribute to the profit.

The complexity and variability occurring in the production process causes challenges. For example, there exist complex interactions of hundreds of factors from different productions steps, which can impact drug quality, yield and production cycle time. *We are interested in end-to-end production process risk*

analysis to control the batch-to-batch variation in yield (protein), quality (impurity), and production cycle time. In system level, we are also interested in total yield, batch pass rate, and key resources utilization. In this paper, we consider the interaction of several decisions, denoted by $\boldsymbol{x}$, impacting on the biomanufacturing system performance, including

1. the releasing policies for upstream cell culture and downstream target protein purification;
2. the threshold values used for systematic quality control after main fermentation process and finished product substance, denoted by (γ_F, γ). After main fermentation or production process, if the impurity percentage is greater than γ_F or γ, the corresponding batch is thrown away;
3. the pooling window for the chromatography process.

Items 1 and 2 are production scheduling and quality control decisions, and the pooling window selection belongs to the operational decision.

3 SIMULATION MODELING

In this section, we develop the stochastic simulation modeling the dynamic evolution of each batch attributes along the production process, which can facilitate the end-to-end biopharmaceutical production risk management and guide the operational decision making. Specifically, pre-culture starts with the biomass attribute, denoted by X_0. Here, we take the inoculum fermentation as a warm-up step and ignore the attribute change before the main fermentation. After the main fermentation, each batch becomes the drug product with attributes, including protein level X and impurity level I, which typically change during centrifuge, chromatography and filtration processes. Thus, in Section 3.1, we present the production system uncertainty quantification to study the batch-to-batch variation. Then, to model the dynamic evolution of quality attributes for each batch through the production process, we explore the bio-chemical knowledge and model the input-output stochastic relationship for each process unit operation including main fermentation, centrifuge, chromatography and filtration in Sections 3.2 and 3.3. Notice that differing with the classical simulation modeling, the probability distributions for each process unit operation are developed to capture cell-level dynamics and model the input-output stochastic relationship for *each batch or entity*.

3.1 Uncertainty Quantification

The simulation output depends on input models and decision variables. Suppose that there are L sources of uncertainty in the biopharmaceutical production process. Denote the input models by $F = \{F_1, \ldots, F_L\}$, where F_ℓ with $\ell = 1, 2, \ldots, L$ quantifies the uncertainty from either raw materials quality or production steps. Denote the decisions involved in the production process by $\boldsymbol{x} = (x_1, x_2, \ldots, x_K)^\top$, including production process parameters, scheduling releasing policy, thresholds of quality control at each step, the pooling window in chromatography, etc. Then, given the input models and decisions $(\boldsymbol{x}, F)$, the batch-to-batch detailed simulation output is $\boldsymbol{Y}(\boldsymbol{x}, F) = \{Y_{ji}(\boldsymbol{x}, F), j = 1, \ldots, J;\ i = 1, \ldots, n\}$, where $\boldsymbol{Y}_j(\boldsymbol{x}, F) = (Y_{j1}(\boldsymbol{x}, F), \ldots, Y_{jn}(\boldsymbol{x}, F))^\top$ is the simulation output from the j-th replication and $Y_{ji}(\boldsymbol{x}, F)$ represents the i-th batch result. Notice $Y_{ji}(\boldsymbol{x}, F)$ could correspond to protein/impurity level, and cycle time.

Given any $(\boldsymbol{x}, F)$, we are interested in analyzing the *steady state batch-to-batch variation* measured by the standard deviation (SD) of simulation detailed output, denoted by $\rho(\boldsymbol{x}, F) = \mathrm{Var}^{1/2}[Y_{ji}(\boldsymbol{x}, F)]$. It is estimated by using the sample variance of simulation batch outputs. Then, the output from j-th replication can be written as

$$\widehat{\rho}_j(\boldsymbol{x}, F) = \rho(\boldsymbol{x}, F) + \varepsilon_j(\boldsymbol{x}, F),$$

where $\widehat{\rho}_j^2(\boldsymbol{x}, F) = \dfrac{1}{n-1} \sum_{i=1}^n \left(Y_{ji}(\boldsymbol{x}, F) - \bar{Y}_j(\boldsymbol{x}, F)\right)^2$, $\bar{Y}_j(\boldsymbol{x}, F) = \dfrac{1}{n} \sum_{i=1}^n Y_{ji}(\boldsymbol{x}, F)$, and $\varepsilon_j(\boldsymbol{x}, F)$ is the corresponding simulation estimation error. We average the estimates from all J replications and obtain the

estimated response,

$$\widehat{\rho}(\boldsymbol{x},F) = \frac{1}{J}\sum_{j=1}^{J}\widehat{\rho}_j(\boldsymbol{x},F). \tag{1}$$

Notice that we use the stationary distribution of $Y_{ji}(\mathbf{x},F)$ as $i \to \infty$ to characterize the variability. Because the number of batches for each product is often very limited in the real biomanufacturing industry, the variance or SD is used to measure the batch-to-batch variation. In addition, we also consider batch mean response $\xi(\boldsymbol{x},F) = \mathrm{E}[Y_{ji}(\boldsymbol{x},F)]$, which can be estimated from the simulation sample mean, $\widehat{\xi}_j(\boldsymbol{x},F) = \frac{1}{n}\sum_{i=1}^{n} Y_{ji}(\boldsymbol{x},F)$, and $\widehat{\bar{\xi}}(\boldsymbol{x},F) = \frac{1}{J}\sum_{j=1}^{J}\widehat{\xi}_j(\boldsymbol{x},F)$. For notation simplification, we only consider one input model here. The underlying "correct" input model, denoted by F^c, is unknown and estimated by m real-world data, denoted by $\boldsymbol{X}_m^{(0)} \equiv \{X_1^{(0)}, X_2^{(0)} \ldots, X_m^{(0)}\}$, with $X_i^{(0)} \overset{i.i.d.}{\sim} F^c$ for $i = 1,2,\ldots,m$. We generate B bootstrapped input model samples quantifying the input model estimation uncertainty as follows.

1. For $b = 1,2,\ldots,B$,
   (1) generate the b-th bootstrap sample of data $\widetilde{\boldsymbol{X}}_m^{(b)} \equiv \{\widetilde{X}_1^{(b)}, \widetilde{X}_2^{(b)} \ldots, \widetilde{X}_m^{(b)}\}$,
   (2) use the b-th data $\widetilde{\boldsymbol{X}}_m^{(b)}$ to obtain corresponding input model estimates $\widehat{F}^{(b)}$,
   (3) run simulation at $(\boldsymbol{x},\widehat{F}^{(b)})$, and get the batch-to-batch variation measure $\widehat{\rho}(\boldsymbol{x},\widehat{F}^{(b)})$ by applying Equation (1).
2. Let $\widehat{R}_b(\boldsymbol{x}) \equiv \widehat{\rho}(\boldsymbol{x},\widehat{F}^{(b)})$ with $b = 1,2,\ldots,B$. We can construct the estimated $(1-\xi)100\%$ two-sided percentile confidence interval (CI) for $\rho(\boldsymbol{x},F^c)$ accounting for both input and simulation uncertainties,
$$\widehat{\mathrm{CI}}(\boldsymbol{x}) = [\widehat{R}_{(\lceil(\xi/2)B\rceil)}(\boldsymbol{x}), \widehat{R}_{(\lceil(1-\xi/2)B\rceil)}(\boldsymbol{x})], \tag{2}$$
   where $\widehat{R}_{(1)}(\boldsymbol{x}) \le \widehat{R}_{(2)}(\boldsymbol{x}) \le \ldots \le \widehat{R}_{(B)}(\boldsymbol{x})$ are order statistics.
3. The impact of input uncertainty can be estimated through the variance,
$$\widehat{\mathrm{Var}}\left[\widehat{R}_b(\boldsymbol{x})\right] = \frac{1}{B-1}\sum_{b=1}^{B}\left(\widehat{R}_b(\boldsymbol{x}) - \bar{R}(\boldsymbol{x})\right)^2 \quad \text{with} \quad \bar{R}(\boldsymbol{x}) = \frac{1}{B}\sum_{b=1}^{B}\widehat{R}_b(\boldsymbol{x}). \tag{3}$$

3.2 Upstream Process (USP) – Cell Culture

Here, we briefly describe the procedure of upstream cell culture process and then discuss each step in USP. For simplification, suppose there is enough external media inventory. The USP starts with the inoculum fermentation. Then, the inoculum would be transfered to main vessel if there is any available resource there. We clean the inoculum tank immediately and start main fermentation at the same time. When the main fermentation is finished, we check the quality of the antigen. If the impurity proportion is higher than a certain threshold γ_F, we throw away this batch. To produce Antigen B that requires the internal media, we start with the media preparation. After that, we clean the mixing vessel immediately and start the inoculum fermentation if the resource is available. The remaining procedure is the same with Antigen A.

Raw Materials and Pre-Culture: To start the production of Antigen A, a batch of pre-culture first seizes an inoculum tank and it takes the fixed processing time; see Table 1. We model the variation of initial biomass X_0 of the pre-culture with a distribution, denoted by F_{X_0} (which could be different for Antigens A and B). This input model is unknown and estimated from the data. To start the production of Antigen B, we seize the mixing vessel and prepare the internal media. When the media preparation is finished, the mixing vessel needs to be cleaned immediately and then released. Notice that media has short lifetime, and needs to be prepared again for main fermentation. If inoculum tank as well as media available, we start the inoculum fermentation. After finished, the inoculum tank needs to be cleaned and released.

Main Fermentation: When the inoculum seizes one main vessel, the main fermentation starts. Otherwise, it waits in the buffer. After the fixed processing time, a new batch of antigen entity is created. Its protein and impurity levels are random functions of exponential-growth-phase time within processing time and the initial bio-mass of pre-culture,

$$X_F = X_0 \cdot e^{\mu T + \varepsilon_P} \quad \text{and} \quad I_F = X_F \cdot \alpha \cdot e^{\varepsilon_I},$$

where $\varepsilon_P \sim N(0, \sigma_P^2)$ and $\varepsilon_I \sim N(0, \sigma_I^2)$ quantify the batch-to-batch variations in protein and impurity levels after fermentation. The exponential phase time $T \sim F_T$ is the effective time that protein grows in fermentation (Doran 1995). It is random and different for each batch. The unknown input model F_T can be estimated from the real-world exponential phase time data $\{T_1, \ldots, T_m\}$ and the unknown parameters $(\mu, \alpha, \sigma_P^2, \sigma_I^2)$ are estimated from the data $(X_{0,i}, X_{F,i}, T_i, I_{F,i})$ with $i = 1, 2, \ldots, m$, where m is the data size. Notice that these parameters can be estimated by using linear regression after taking log-transformation. After a batch of antigen is generated, we discard it if its impurity percentage is greater than the pre-determined threshold γ_F. Otherwise, the antigen will be stored in the freezing buffer before released to the downstream processes. Again a cleaning process would start right after the main fermentation. Notice that if γ_F is set too high, we may waste the DSP resource on producing those hopeless batches. If it is too low, we may discard some profitable batches.

3.3 Downstream Process (DSP) – Target Protein Purification

The DSP starts with the centrifuge (it includes the inactivation). Since antigens are fragile and require the "non-wait" constraint, which prevents the antigen wait during the whole DSP. Thus, the DSP releasing police is a critical decision for the biomanufacturing process. Since the chromatography usually takes the most time, we can start with a policy that releases the antigen when the previous batch finishes the chromatography process. Specifically, in the DSP, a batch of antigen goes to the centrifuge station first, seizes an equipment, and delays a fixed processing time; see Table 1. The centrifuge does not change the protein level, but removes a random portion of impurity; see Equation (4). When we finish this batch, the centrifuge equipment goes to cleaning process with fixed cleaning time, and then it is released. After the centrifuge, antigen goes to the chromatography process which also seizes an equipment and delays the fixed processing time; see Table 1. Each chromatograph step removes a random proportion of protein and impurity; see Equation (5). Based on the resulting impurity proportion, if it is greater than γ, we repeat chromatography (need switch antigen to another equipment). Suppose that γ is the FDA quality requirement for Antigens A and B. *Thus, the number of chromatograph steps is random (which depends on the batch quality) with the certain upper limit, say M.* After each step, the corresponding equipment needs to be cleaned and then released. If the resulting impurity proportion cannot be reduced to $\leq \gamma$ after M steps, this batch is discarded.

Then, the antigen goes to the filtration process, seizes an equipment and delays a fixed processing time. Filtration slightly reduces the impurity level; see Equation (6). When the filtration is finished, the equipment goes to the cleaning procedure and is released after a fixed cleaning time. After that, the antigen goes to the quality control process, which takes a fixed testing time to check the quality of the finished API. The final impurity proportion must be no greater than the quality requirement γ.

Centrifuge: The protein and impurity levels before and after centrifuge are denoted by (X_F, I_F) and (X_C, I_C). We can assume this step does not change the protein, i.e. $X_C \equiv X_F$ (Delahaye et al. 2015), and it removes random proportion of impurity,

$$I_C = Q \cdot I_F. \tag{4}$$

The distribution of random ratio Q could be estimated by using data $(I_{F,i}, I_{C,i})$ with $i = 1, 2, \ldots, m$. In the case study, we assume that Q follows Uniform distribution $Q \sim \text{Unif}(0.4, 0.5)$; see Leung (2007), Gottschalk (2009), Roush and Lu (2008).

Table 1: Fixed processing time for each step.

Time (hours)	Antigen A	Clean Equipment
Inoculum Fermentation	24	1.5
Media Preparation	—	0.6
Main Fermentation	72	6.0
Centrifuge	2.5	0.2
Chromatography	8.0	1.5
Filtration	2.0	0.5
Quality Control	2.0	—

Chromatography: The protein and impurity levels of antigen before and after each chromatography step are denoted by (X_C, I_C) and (X_P, I_P), given the pooling window w. Chromatography techniques rely on difference in physical-chemical characteristics between proteins and impurities to separate one from other. The output lanes would have different proportions of protein and impurities. A pooling window specified by the starting/ending lanes and chromatography technique expects to "pool out" the part containing more protein and less impurities. *The pooling window is an operational decision that could differ for each batch and each step.* In our case study, we adopt the 3-step pooling window policy in Martagan et al. (2017). Given a pooling window, each chromatograph step removes random proportions of protein and impurity,

$$X_P = (Q_P|w) \cdot X_C \quad \text{and} \quad I_P = (Q_I|w) \cdot I_C; \tag{5}$$

see Martagan et al. (2017). Given the data $Q_{P,i}$, $Q_{I,i}$ and w_i for $i = 1, 2, \ldots, m$ from each chromatography step, we need to estimate the distributions of random ratios $Q_P|w$ and $Q_I|w$.

Filtration: Denote the antigen protein and impurity levels before and after filtration with (X_P, I_P) and (X_{fr}, I_{fr}). Filtration works as a polishing procedure. It only slightly reduces the impurity,

$$I_{fr} = \quad Q_{fr} \cdot I_P, \tag{6}$$

and does not change the protein level, $X_{fr} = X_P$. In the case study, we assume that the random ratio Q_{fr} follows a Uniform distribution, $Q_{fr} \sim \text{Unif}(0.99, 1)$, see Leung (2007), Gottschalk (2009), Roush and Lu (2008).

4 AN ANTIBODY PRODUCTION CASE STUDY

In this case study, due to the limitation of our data, we consider the value stream that only produces Antigen A. The resources of bio-drug substance production system in Figure 1 include the equipments needed in each step, i.e., inoculum tank [5], mixing vessel [5], main vessel [5], centrifuge equipment [2], chromatography [5] and filtration equipments [2], where the number in the bracket $[\cdot]$ gives the capacity or the number of equipments at each working station. One batch at each production step only requires one corresponding equipment. For simplification, suppose each production step requires a fixed processing time (in hours) as given in Table 1. We need to clean up the equipment immediately after production and the cleaning time for each equipment is also provided in Table 1.

4.1 Input Modeling and Uncertainty Quantification

In this section, we provide the input modeling procedure for each key source of uncertainty in biomanufacturing production process. We further describe the detailed procedure to fit the corresponding input models given the real-world data.

Raw Materials and Pre-Culture: We have collected the starting biomass data $\{X_{0,1}, X_{0,2}, \ldots, X_{0,m}\}$ of pre-culture and also main fermentation, where $X_{0,i} \overset{i.i.d.}{\sim} F_{X_0}$ for $i = 1, 2, \ldots, m$. We consider the nonparametric empirical distribution, $\widehat{F}_{X_0}(x) = \frac{1}{m}\sum_{i=1}^{m} \mathrm{I}(X_{0,i} \leq x)$, and then use the bootstrap to quantify the input uncertainty

Main Fermentation: The data available for main fermentation include starting biomass $X_{0,i}$, exponential phase time T_i, and ending protein level $X_{F,i}$, with $i = 1, 2, \ldots, m$. Similarly, we use the empirical distribution to model the randomness of exponential phase time, $\widehat{F}_T(t) = \frac{1}{m}\sum_{i=1}^{m} \mathrm{I}(T_i \leq t)$. For the protein model in main fermentation step, we can fit the linear regression,

$$\log X_F - \log X_0 = \mu T + \varepsilon_P,$$

and the parameter μ can be estimated by least-square, $\widehat{\mu} = \min_{\mu} \sum_{i=1}^{m} (\log X_{F,i} - \log X_{0,i} - \mu T_i)^2$. Denote the residuals $r_i = \log X_{F,i} - \log X_{0,i} - \widehat{\mu} T_i$. Then, σ_P^2 can be estimated by residual variance, $\widehat{\sigma}_P^2 = \sum_{i=1}^{m}(r_i - \bar{r})^2/(m-1)$, where $\bar{r} = \sum_{i=1}^{m} r_i/m$.

Since we do not have the corresponding impurities data $I_{F,i}$, we set the coefficient $\alpha = 1.5$ following Martagan et al. (2017), and assume $\sigma_I = \beta_0 \sigma_P$. We would further study the sensitivity over the selection of β_0 in Section 4.2.

Centrifuge: Notice that we do not have the corresponding data $I_{F,i}, I_{C,i}$, and also it is not a very critical process. We directly apply a Uniform distribution for random proportion Q, that $Q \sim \mathrm{Unif}(0.4, 0.5)$ according to expert knowledge (Leung 2007; Gottschalk 2009; Roush and Lu 2008).

Chromatography: For each chromatography step, instead of having exact data for (Q_P, Q_I, w), we have the mean proportions of protein and impurity for various candidate pooling window, denoted by $q_P(w)$ and $q_I(w)$, where $w \in S_w$ and $S_w = \{w_1, w_2, \ldots, w_K\}$ is the set of candidate pooling window. We apply the same distributional model for chromatography as Martagan et al. (2017), which is uniform distribution within 10% of their mean for all $w \in S_w$,

$$Q_P|w \sim \mathrm{Unif}(0.9q_P(w), 1.1q_P(w)) \quad \text{and} \quad Q_I|w \sim \mathrm{Unif}(0.9q_I(w), 1.1q_I(w)).$$

Filtration: We do not obtain the data for filtration step, and also it is not a critical step. We assume the random proportion following uniform $Q_{fr} \sim \mathrm{Unif}(0.99, 1)$ according to expert knowledge (Leung 2007; Gottschalk 2009; Roush and Lu 2008).

We first fit the input model for fermentation protein level. From the $m = 20$ batches real-world data $\{(X_{0,i}^{(0)}, X_{F,i}^{(0)}, T_i^{(0)}),\ i = 1, \ldots, m\}$, we fit the no-intercept linear regression and obtain the corresponding point estimates $\widehat{\mu} = 0.0475$ and $\widehat{\sigma}_P = 0, 0.4918$, with Adjusted R-square 0.9656 and p-value 1.392E-15, which implies,

$$X_F = X_0 \cdot e^{0.0475T + \varepsilon_P}, \quad \varepsilon_P \sim N(0, 0.4918^2).$$

We further perform the KolmogorovSmirnov (K-S) test on the residuals with fitted normal distribution, which gives the test statistics D=0.12954, and p-value 0.8485. Thus, we claim that under current data, we statistically do not reject this model.

Since the input models $F = \{F_{X_0}, F_T, \mu, \sigma_P\}$ are estimated by $m = 20$ real-world data $\{(X_{0,i}^{(0)}, X_{F,i}^{(0)}, T_i^{(0)}),\ i = 1, \ldots, m\}$, where F_{X_0} is for raw material, F_T is for exponential phase time, and (μ, σ_P) for main fermentation protein model. We consider non-parametric bootstrap approach describe in Section 3.1 to quantify the input model estimation uncertainty, and we generate $B = 1000$ bootstrap samples. For each bootstrap data sample, we fit corresponding input models and run $J = 100$ replication, and run-length $n = 200$, after a warm-up of 500 batches. By applying Equations (2) and (3), for the batch-to-batch mean and SD response of protein/impurity level and cycle time, we record the 95% percent CI lower/upper bounds and bootstrap variance in Table 2. Based on that, the limited data (20 batches) does have big impact of simulation

estimation uncertainty. In order to more accurately quantify the batch-to-batch variation, more real-world data should be collected.

Table 2: Quantifying impact of input estimation uncertainty to the simulation output.

Output	Mean Protein	Mean Impurity	Mean CycleTime	SD Protein	SD Impurity	SD CycleTime
CI Lower	104.69	9.89	166.19	84.31	7.91	35.44
CI Upper	171.33	15.82	174.13	184.28	16.54	41.36
Variance	308.46	2.39	4.20	594.43	4.58	2.22

We verify the simulation model under simplified situations. We first study the output trend to verify whether the proposed simulation matches the intuition. Then, we separately consider USP and DSP, and make some simplifications so that the model of production process becomes a Jackson Network (JN): (1) modify the inter-arrivals and processing times following certain exponential distributions; (2) let the cleaning time of all equipments to be zero; and (3) set the number of chromatography steps to be one. We compare the analytical expected cycle time with simulation estimates to validate the simulation model. Under this assumption, USP is a JN with two $M/M/c$ queues (inoculum and fermentation), and DSP is a JN with three $M/M/c$ queues (centrifuge, chromatography and filtration) and one $M/M/\infty$ queue (quality control). From the simplified simulation experiments, the analytical mean cycle time of USP $T_{USP} = 1.067$ and DSP $T_{DSP} = 2.067$, matches the simulation estimated cycle time 95% CI for USP $[1.060, 1.072]$ and DSP $[2.062, 2.076]$.

4.2 Sensitivity Analysis of Impurities Variation in Main Fermentation

We perform the sensitivity analysis of system performance at different levels of $\beta_0 = \sigma_I/\sigma_P$. We run simulation at different β_0 and estimate the batch-to-batch SD for protein $\widehat{\rho}^{(X)}(\boldsymbol{x}, F; \beta_0)$, impurities $\widehat{\rho}^{(I)}(\boldsymbol{x}, F; \beta_0)$, and cycle time $\widehat{\rho}^{(t)}(\boldsymbol{x}, F; \beta_0)$ respectively, where (X), (I) and (t) represent the corresponding simulation outputs. We also record the batch-to-batch mean response estimate $\widehat{\xi}^{(X)}(\boldsymbol{x}, F; \beta_0)$, $\widehat{\xi}^{(I)}(\boldsymbol{x}, F; \beta_0)$, and $\widehat{\xi}^{(t)}(\boldsymbol{x}, F; \beta_0)$. In this section, we consider three levels $\beta_0 = 0.5, 1, 1.5$ to study the impact of relative impurities variation on the output. For each level, we run $J = 100$ replication, and run-length $n = 200$, after a warm-up of 500 batches. In addition to batch-based simulation output , we also look at system level output as follows.

- total yield of 200 batches, which sums up the proteins of all finished and qualified batches, $W_j(\boldsymbol{x}, F; \beta_0) = \sum_{i=1}^{n} X_{ji} \mathbb{I}((j,i) \in FQ)$, where $FQ = \{(j,i)$ finished & qualified$I_{ji}/(X_{ji} + I_{ji}) \leq \gamma\}$ and $\mathbb{I}(\cdot)$ is the indicator function, we average over replications $\bar{W}(\boldsymbol{x}, F; \beta_0) = \frac{1}{J} \sum_{j=1}^{J} W_j(\boldsymbol{x}, F; \beta_0)$.
- batch pass rate, the proportion of batches that finish and are qualified, $\eta_j(\boldsymbol{x}, F; \beta_0) = \frac{1}{n} \sum_{i=1}^{n} \mathbb{I}((j,i) \in FQ)$, and then $\bar{\eta}(\boldsymbol{x}, F; \beta_0) = \frac{1}{J} \sum_{j=1}^{J} \eta_j(\boldsymbol{x}, F; \beta_0)$.
- equipment utilizations of inoculum, fermentation, and chromatography.

The results in Table 3 indicate the system performance is similar for $\beta_0 = 0.5, 1, 1.5$. As β_0 increases, we can observe: (1) the impurities variation increases; (2) more batches are dropped after main fermentation; (3) the amount of total protein and pass rate decrease; (4) the average cycle time has slight decrease; and (5) the utilization of chromatography decreases.

4.3 Performance Analysis under Different Decisions

In this section, we compare different decision scenarios for scheduling, quality control and pooling window operational decisions discussed in Section 2.

Table 3: Sensitivity analysis of system performance at $\beta_0 = 0.5, 1, 1.5$.

β_0	0.5	1	1.5
Mean Protein $\widehat{\xi}^{(X)}(\boldsymbol{x}, F; \beta_0)$	133.445±0.946	133.632±0.952	139.113±1.187
Mean Impurity $\widehat{\xi}^{(I)}(\boldsymbol{x}, F; \beta_0)$	12.475±0.085	12.414±0.089	12.615±0.112
Mean CycleTime $\widehat{\xi}^{(t)}(\boldsymbol{x}, F; \beta_0)$	154.465±1.518	151.368±1.551	148.679±1.591
SD Protein $\widehat{\rho}^{(X)}(\boldsymbol{x}, F; \beta_0)$	125.42±2.094	127.825±2.264	134.585±2.487
SD Impurity $\widehat{\rho}^{(I)}(\boldsymbol{x}, F; \beta_0)$	11.874±0.216	11.678±0.206	11.916±0.237
SD CycleTime $\widehat{\rho}^{(t)}(\boldsymbol{x}, F; \beta_0)$	35.943±1.463	36.456±1.623	35.188±1.302
Yield $\bar{W}(\boldsymbol{x}, F; \beta_0)$	24799±183	22660±188	21501±207
Pass Rate $\bar{\eta}(\boldsymbol{x}, F; \beta_0)$	0.929±0.002	0.847±0.002	0.772±0.003
Inoculum Utilization	0.211±0.001	0.209±0.001	0.21±0.001
Fermentation Utilization	0.652±0.004	0.646±0.004	0.65±0.004
Chromatography Utilization	0.209±0.001	0.176±0.001	0.148±0.001

USP/DSP Releasing: The upstream releasing policy is based on the total number of working batches in the upstream, denoted by N_U. Since chromatography dominants the downstream time, the downstream releasing policy is based on total number of working batches in the centrifuge and chromatography steps, denoted by N_D. We consider three scenarios $(N_U, N_D) = (4,2),\ (5,2),\ (8,4)$ that correspond to slow to fast releasing policy.

Pooling Window: We consider the pooling windows $(w^{(1)}, w^{(2)}, w^{(3)})$, where $w^{(i)}$ is pooling window of i-th step chromatography. The pooling window selection is dynamic, batch- and state-based, which depends on the protein and impurity levels. For simplification, here we directly apply the optimal pooling policy in Martagan et al. (2017). We would simultaneously optimize the pooling window, production scheduling and quality control policies in our future extension.

Impurity Proportion Threshold: We consider a fixed final quality requirement $\gamma = 15\%$. The impurity threshold for main fermentation quality control is an operational decision. Given the i-th batch protein level and impurity level $(X_{F,i}, I_{F,i})$, we have fixed pooling policy $(w_i^{(1)}, w_i^{(2)}, w_i^{(3)})$ for each chromatography step. According to $Q_P|w \sim \text{Unif}(0.9q_P(w), 1.1q_P(w))$ and $Q_I|w \sim \text{Unif}(0.9q_I(w), 1.1q_I(w))$ for each chromatograph, we are able to compute most, expected and least protein outcome proportion of starting protein level after DSP purification as $u_{\max,i} = X_{\max,i}/X_{F,i} = \prod_{k=1}^{3} 1.1q_P(w_i^{(k)})$, $u_{exp,i} = X_{exp,i}/X_{F,i} = \prod_{k=1}^{3} q_P(w_i^{(k)})$ and $u_{\min,i} = X_{\min,i}/X_{F,i} = \prod_{k=1}^{3} 0.9q_P(w_i^{(k)})$ respectively. Then, since $Q \sim \text{Unif}(0.4, 0.5)$ for centrifuge, we can further compute most, expected and least impurities proportion of starting impurity level after purification $v_{\max,i} = I_{\max,i}/I_{F,i} = 0.5\prod_{k=1}^{3} 1.1q_I(w_i^{(k)})$, $v_{exp,i} = I_{exp,i}/I_{F,i} = 0.45\prod_{k=1}^{3} q_I(w_i^{(k)})$ and $v_{\min,i} = 0.4I_{\min,i}/I_{F,i} = \prod_{k=1}^{3} 0.9q_I(w_i^{(k)})$, where 0.5, 0.45 and 0.4 are most, expected and least proportion in centrifuge. We assume three γ_F policy that discard the batch based on best, expected, and worst scenarios in the following purification steps. Thus, by solving $\frac{\gamma_F v_{\min,i}}{(1-\gamma_F)u_{\max,i}+\gamma_F v_{\min,i}} = \gamma$, we can get the best case $\gamma_F^{best} = \gamma u_{\max,i}/(v_{\min,i} + \gamma u_{\max,i} - \gamma v_{\min,i})$. Similarly, we can get the expected case $\gamma_F^{exp} = \gamma u_{exp,i}/(v_{exp,i} + \gamma u_{exp,i} - \gamma v_{exp,i})$ and the worst case $\gamma_F^{worst} = \gamma u_{\min,i}/(v_{\max,i} + \gamma u_{\min,i} - \gamma v_{\max,i})$. Therefore, we can select the γ_F based on the best to worst scenario which represents the quality policy with the conservative level from low to high.

At each decision setting, we run $J = 100$ replications and each simulation run has warmup- and run-length equal to 500 and 200 batches. The experiments results with different USP/DSP scheduling and quality control decisions are shown in Table 4 and 5. In terms of USP/DSP releasing policy, we can see that it mainly influences the average cycle time and batch variations on cycle time. The equipment utilization only slightly increases because the utilization mainly depends on the arrival rate, processing time and capacity at each step. As (N_U, N_D) increases, the time from order arrival to starting production

decreases and so does its variation, which contributes to the overall cycle time reduction. On the other hand, since increasing N_D could lead to "no wait" constraint violation, more batches are throw away in DSP. So we observe some decrease in total protein and pass rate.

Table 4: Production system performance at different USP/DSP scheduling policies.

(USP, DSP)	(4, 2)	(5, 2)	(8, 4)
Mean Protein $\widehat{\xi}^{(X)}(\boldsymbol{x},F)$	134.17±1.10	133.63±0.95	132.95±0.93
Mean Impurity $\widehat{\xi}^{(I)}(\boldsymbol{x},F)$	12.543±0.099	12.414±0.089	12.455±0.084
Mean CycleTime $\widehat{\xi}^{(t)}(\boldsymbol{x},F)$	581.03±30.27	151.36±1.55	130.91±0.48
SD Protein $\widehat{\rho}^{(X)}(\boldsymbol{x},F)$	128.75±2.23	127.82±2.26	128.02±2.10
SD Impurity $\widehat{\rho}^{(I)}(\boldsymbol{x},F)$	11.912±0.22	11.678±0.206	11.778±0.194
SD CycleTime $\widehat{\rho}^{(t)}(\boldsymbol{x},F)$	125.49±5.70	36.45±1.62	16.60±0.63
Yield $\bar{W}(\boldsymbol{x},F)$	22722±200	22660±188	22314±175
Pass Rate $\bar{\eta}(\boldsymbol{x},F)$	0.847±0.003	0.847±0.002	0.839±0.002
Inoculum Utilization	0.205±0.001	0.209±0.001	0.209±0.001
Fermentation Utilization	0.634±0.002	0.646±0.004	0.647±0.004
Chromatography Utilization	0.172±0.001	0.176±0.001	0.175±0.001

For the main fermentation QC threshold γ_F changing from best to worst scenario, we tend to throw away more batches after the fermentation step; see Table 5. Consequently, for batch level, we can increase the average protein. However, it might not be profitable as we are getting less batches. Notice the total protein and pass rate reduced a lot. The chromatography equipment utilization also decreases in the worst scenario case since less batches would be able to enter the DSP.

Table 5: Production system performance at different QC threshold levels γ_F after main fermentation.

γ_F	best scenario	expected scenario	worst scenario
Mean Protein $\widehat{\xi}^{(X)}(\boldsymbol{x},F)$	135.396±0.984	133.632±0.952	145.623±1.359
Mean Impurity $\widehat{\xi}^{(I)}(\boldsymbol{x},F)$	12.596±0.087	12.414±0.089	13.02±0.123
Mean CycleTime $\widehat{\xi}^{(t)}(\boldsymbol{x},F)$	152.64±1.711	151.368±1.551	149.368±1.459
SD Protein $\widehat{\rho}^{(X)}(\boldsymbol{x},F)$	132.326±2.754	127.825±2.264	139.371±3.222
SD Impurity $\widehat{\rho}^{(I)}(\boldsymbol{x},F)$	12.109±0.271	11.678±0.206	12.742±0.308
SD CycleTime $\widehat{\rho}^{(t)}(\boldsymbol{x},F)$	36.883±1.558	36.456±1.623	35.57±1.292
Yield $\bar{W}(\boldsymbol{x},F)$	23243±177	22660±188	17978±198
Pass Rate $\bar{\eta}(\boldsymbol{x},F)$	0.859±0.002	0.847±0.002	0.617±0.004
Inoculum Utilization	0.21±0.001	0.209±0.001	0.21±0.001
Fermentation Utilization	0.648±0.004	0.646±0.004	0.65±0.004
Chromatography Utilization	0.181±0.001	0.176±0.001	0.119±0.001

5 CONCLUSIONS

In this paper, we develop the stochastic simulation for the biopharmaceutical production process from raw materials to finished drug substance. This model can integrate key sources of uncertainties from USP/DSP. Thus, it can support the end-to-end production process risk management. We explore the biotechnology domain knowledge as well as queueing network modeling in the simulation model. The case study demonstrates that the proposed simulation model can study the system risk analysis and guide the development of efficient and stable biopharma production process.

REFERENCES

Delahaye, M., K. Lawrence, S. Ward, and M. Hoare. 2015. "An Ultra Scale-Down Analysis of the Recovery by Dead-End Centrifugation of Human Cells for Therapy". *Biotechnology and bioengineering* 112(5):997–1011.

Doran, P. M. 1995. *Bioprocess Engineering Principles*. Amsterdam: Elsevier.

Fleischhacker, A. J., and Y. Zhao. 2011. "Planning for Demand Failure: A Dynamic Lot Size Model for Clinical Trial Supply Chains". *European Journal of Operational Research* 211(3):496–506.

Gottschalk, U. 2009. *Process Scale Purification of Antibodies*. Number 577.27 PRO. Hoboken: John Wiley & Sons.

Kulkarni, N. S. 2015. "A Modular Approach for Modeling Active Pharmaceutical Ingredient Manufacturing Plant: A Case Study". In *Proceedings of the 2015 Winter Simulation Conference*, edited by L. Yilmaz et al., 2260–2271. Piscataway, New Jersey: Institute of Electrical and Electronics Engineers, Inc.

Lakhdar, K., and L. G. Papageorgiou. 2008. "An Iterative Mixed Integer Optimisation Approach for Medium Term Planning of Biopharmaceutical Manufacture under Uncertainty". *Chemical Engineering Research and Design* 86(3):259–267.

Leachman, R. C., L. Johnston, S. Li, and Z.-J. Shen. 2014. "An Automated Planning Engine for Biopharmaceutical Production". *European Journal of Operational Research* 238(1):327–338.

Leung, W. W.-F. 2007. *Centrifugal Separations in Biotechnology*. Amsterdam: Elsevier.

Lim, A. C., Y. Zhou, J. Washbrook, N. J. Titchener-Hooker, and S. Farid. 2004. "A Decisional-Support Tool to Model the Impact of Regulatory Compliance Activities in the Biomanufacturing Industry". *Computers & Chemical Engineering* 28(5):727–735.

Martagan, T., A. Krishnamurthy, and P. Leland. 2018. "Managing Trade-offs in Protein Manufacturing: How Much to Waste?". *Manufacturing & Service Operations Management*.

Martagan, T., A. Krishnamurthy, P. A. Leland, and C. T. Maravelias. 2017. "Performance Guarantees and Optimal Purification Decisions for Engineered Proteins". *Operations Research* 66(1):18–41.

Martagan, T., A. Krishnamurthy, and C. T. Maravelias. 2016. "Optimal Condition-Based Harvesting Policies for Biomanufacturing Operations with Failure Risks". *IIE Transactions* 48(5):440–461.

Nfor, B. K., P. D. Verhaert, L. A. van der Wielen, J. Hubbuch, and M. Ottens. 2009. "Rational and Systematic Protein Purification Process Development: the Next Generation". *Trends in Biotechnology* 27(12):673–679.

Otto, R., A. Santagostino, and U. Schrader. 2014. "From Science to Operations: Questions, Choices, and Strategies for Success in Biopharma". *Mc Kinsey*.

Roush, D. J., and Y. Lu. 2008. "Advances in primary recovery: centrifugation and membrane technology". *Biotechnology progress* 24(3):488–495.

Saraph, P. V. 2001. "Biotech Industry: Simulation and Beyond". In *Proceedings of the 2001 Winter Simulation Conference*, edited by B. A. Peters et al., Volume 2, 838–843. Piscataway, New Jersey: Institute of Electrical and Electronics Engineers, Inc.

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