Bioreactor Fill Process Control Using Inline Concentration Measurement

by

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ABSTRACT

Some biopharmaceutical companies have responded to evolution of the competitive landscape by placing additional emphasis on reducing their costs of manufacturing as a means of maintaining competitiveness. The prototypical current generation biopharmaceutical drug substance manufacturing facility requires a large upfront capital investment. Improving efficiency of use of existing facilities, such as by improving production throughput through the adoption of technology, represents one way in which a company may reduce its costs of manufacturing and/or avoid or delay investments in additional capacity needed to meet future demand.

Reducing the variability in the performance of a liquid filling operation taking place during the protein production step is desirable, because it: (1) enables process optimization, including potential throughput expansion, (2) demonstrates control over the process, and (3) improves step yield reproducibility. The technical and economic bases for the implementation of an alternative process control strategy intended to reduce this variability are presented. This strategy involves controlling the fill operation using an inline concentration measurement of the parameter of interest. An engineering-probabilistic approach, consisting of a transient concentration profile model built into a Monte Carlo framework, is applied to predict the variability of the performance of a concentration-based control strategy for filling an agitated, gassed bioreactor. An optimization methodology for selecting an appropriate post-fill target concentration and for quantifying the economic benefit of reducing variability is proposed.

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NOTE ON PROPRIETARY INFORMATION

Information used in the analysis described in this document that is proprietary to Amgen is withheld. Such information includes, but is not limited to, the raw data used to develop the transient concentration profile model and the Monte Carlo simulation described in Chapters 5 and 6, respectively.

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List of Abbreviations

ARI	Amgen Rhode Island
CCP	Cell Culture Parameter
CFD	Computational Fluid Dynamics
cGMP	Current Good Manufacturing Practices
CHO	Chinese Hamster Ovary
KL Model	Khang-Levenspiel Model
RHS	Right-Hand Side
RTD	Residence Time Distribution
SKL Model	Summed Khang-Levenspiel Model
VBA	Visual Basic for Applications

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1. INTRODUCTION

The biopharmaceutical industry delivers therapeutic proteins to patients. Many of these medications are indicated for the treatment of grievous illness and serve a critical function in the management of these diseases. These molecules are often manufactured through a batch process in which the desired protein is expressed using a genetically engineered cell line such as Chinese Hamster Ovary (CHO) or *E. coli* cultured in a large bioreactor and then purifying and formulating it for use by patients [1]. The performance of biopharmaceutical manufacturing processes may be enhanced with respect to a number of objectives, for example, yield or cycle time. Such enhancements are critical to a facility's ability to supply consistent and efficacious material in a cost effective manner.

A discussion of an improvement intended to enhance the consistency of the cell culture process by which the protein of interest is produced constitutes the focus of this document. This process improvement involves the use of an alternative process control strategy that would be intended to reduce the variability of the performance of a batch filling operation associated with the protein production step. While the focus of this document is restricted to a particular cell culture operation, the methodologies developed through this work may be generally applied to: (1) batch chemical injection operations conducted in agitated tanks, and (2) quantification of the economic impact of the variability inherent in these operations.

1.1. PROJECT MOTIVATION

The profit margins that biopharmaceutical companies have historically been able to earn have been supported by patent exclusivity and a number of challenges that limit the competitive threat posed by follow-on biologics [2]. Some companies have responded to expected evolution of the competitive landscape by placing additional emphasis on reducing their costs of manufacturing as a means of protecting their competitiveness.

The prototypical current generation biopharmaceutical drug substance manufacturing facility requires a large upfront capital investment [3]. Making more efficient use of its existing

[1] Shuler and Kargı, *Bioprocess Engineering / Michael Shuler, Fikret Kargi.*
[2] Pasanek, "The Conclusion of a Biologic's Lifecycle."
[3] "Amgen Announces FDA Licensure of Two New Manufacturing Facilities; Company Continues to Meet Increased Demand for Its Novel Therapeutics."

production facilities represents one way in which a company may reduce its costs of manufacturing. Specifically, by using existing plants more efficiently, companies may be able to avoid or delay investments in additional production capacity and the associated capital and fixed costs that it might otherwise need to make to meet future demand. These efficiency gains may be realized by improving one or more of the following aspects of the production processes for its commercial and clinical molecules: (1) yield (mass of protein per production lot), (2) run rate (production lots per time), and/or (3) success rate (probability a given production run will yield in-specification product). Introducing a new technology into an existing facility represents a possible means of gaining yield, run rate, and/or success rate improvements.

1.2. Problem Statement

The purpose of the internship, during which the work providing the basis for this document was performed, was to identify technologies that would provide yield, run rate, and/or success rate improvements in an existing biopharmaceutical manufacturing facility and to develop business cases to support the potential adoption of promising candidates. The technical and economic bases for the implementation of one of these technologies are described in this document.

During the protein production step, various liquids, such as cell culture media, suspension containing live cells, and other nutrients, are added to the Production Reactor. Variability inherent in a particular filling operation executed during this step introduces a finite probability that the post-fill value of a cell culture parameter (CCP) realized in the Production Reactor culture will fall outside of pre-defined control limits during a given run, violating the process specification. Reducing this variability is desirable, because: (1) it demonstrates control over the process, (2) improves step yield reproducibility, and (3) reduces the probability of violating control limits and the associated business and potential plant quality impacts. To protect proprietary information, the nature of this CCP is not discussed in this document. Sufficient information is provided in the body of the text such that the nature of the CCP does not affect the analysis described in this document.

1.3. PROJECT GOALS

The first goal of this project is to predict the extent to which variability in the filling operation may be reduced by controlling the process using an inline measurement of CCP concentration in the Production Reactor rather than the current volume-transfer-based strategy. The second goal is to establish a risk-based framework for use in selecting a target value for the post-fill CCP concentration and quantifying the economic impact of this process improvement.

The purpose of conducting this work is twofold. First, these goals directly support the development of a business case for the process improvement of interest. Second, the risk-based framework may be adapted as needed to other process decisions in which stochastic effects are of practical importance.

1.4. PROJECT APPROACH

The content of this document is based on work performed during a 6.5-month internship completed at the Amgen Rhode Island (ARI) drug substance manufacturing facility. As described in Section 1.2, the purpose of this internship was to identify technologies that could provide a throughput benefit and develop supporting business cases for promising candidates. This work was conducted in three phases: (1) analysis of the current capabilities of the facility, (2) identification of technologies that may be used to improve process throughput, and (3) assessment of the technical and economic feasibility of implementing promising candidate technologies. The proposed cell culture process control strategy, which is the focus of this document, was identified through the current state assessment of the facility. The assessment of its technical and economic feasibility of implementation forms the content of this document. Additional description of the identification, selection, and business case development for other technologies considered as part of the internship is outside the scope of this document and is not discussed further.

The performance of the proposed process control strategy, hereafter referred to as concentration-based control, is predicted using an engineering and probabilistic modeling framework. This framework consists of an engineering model that is used to simulate the transient mixing process taking place in the Production Reactor during the fill and a Monte Carlo simulation that is used to account for the variability inherent in the system. Additional discussion of this framework is provided in the body of this document. An engineering-probabilistic

approach is considered appropriate for this application for the following reasons. First, physically simulating the variabilities of interest in a representative manner would require conducting experiments in the Production Reactors, which is both cost and time prohibitive. Second, a probabilistic approach is considered appropriate given the prominence of stochastic effects in the problem of interest and the capability of this approach to generate a statistically robust prediction of process variability.

A risk-based framework that may be used to quantify the economic benefit of the improved performance provided by concentration-based control is developed for the problem of interest. In addition to its use in assessing concentration-based control, this framework may also be used to support recommendations regarding changes to the fill operating strategy.

1.5. THESIS STATEMENT

The thesis proposed in this document consists of two parts. First, it is asserted that under the conditions of interest, the variability of the performance of the filling operation may be reduced by implementing a concentration-based process control strategy in place of the current volume-transfer-based strategy. This assertion is supported by a prediction of the variability of this control strategy made using a Monte Carlo framework developed using historical operating data. Second, it is asserted that the benefit of reduced variability may be quantified using a risk-based framework. An example is provided to demonstrate the application of this framework.

1.6. THESIS OVERVIEW

The discussion provided in this document is organized into a number of chapters and sub-sections, which are briefly summarized as follows.

Chapter 1 – The purpose of Chapter 1is to provide an introduction to this document. Included in this chapter are the: (1) project motivation, (2) problem statement, (3) project goals, (4) project approach, (5) thesis statement, and (6) thesis overview.

Chapter 2 – The purpose of Chapter 2 is to provide a brief description of the Amgen drug substance manufacturing network and some specifics of Production Reactor filling at ARI as a means of establishing context for the work described in this document.

Chapter 3 – The purpose of Chapter 3 is to provide a basis for the modeling work described in Chapters 5 through 7. Included in this chapter are the following: (1) a summary of

the rationale for high-level aspects of the modeling approach, and (2) a review of modeling of transient concentration profiles encountered during batch chemical injections performed in mixed tanks as documented in the literature.

Chapter 4 – The purpose of Chapter 4 is to provide a brief description of the current state of the Production Reactor filling operation of interest to establish a basis against which the predicted variability of concentration-based control may be compared.

Chapter 5 – The purpose of Chapter 5 is to provide a description of the model developed to simulate the transient CCP concentration profile in the Production Reactor during the fill. Included in this chapter are descriptions of the: (1) data used to determine the model parameters, (2) transient concentration profile model development, (3) model fitting approach, and (4) modeling results. This model is used in the Monte Carlo simulation described in Chapter 6 to provide a simulation of the transient CCP concentration profile in the Production Reactor during the filling operation.

Chapter 6 – The purpose of Chapter 6 is to provide a description of the probabilistic simulation developed to predict the performance of the concentration-based control strategy. Included in this chapter are descriptions of the: (1) probabilistic modeling methodology, (2) probabilistic model inputs and model baselining, (3) results of the simulation, and (4) recommended process control strategy. The results of the probabilistic simulation serve as an input in the example application of the optimization method described in Chapter 7.

Chapter 7 – The purpose of Chapter 7 is to provide an optimization framework for selecting operating setpoints when variability is of practical importance and for quantifying the value of improvements that reduce process variability. Included in this chapter are: (1) a description of the optimization methodology for selecting the target CCP concentration, (2) an example application of this methodology, and (3) a description of how the methodology may be applied to quantify the benefit provided by reducing variability.

Chapter 8 – The purpose of Chapter 8 is to provide a summary of the conclusions and recommendations outlined in this document. Included in this chapter are: (1) a summary of conclusions related to the Production Reactor filling operation at ARI, and (2) a list of recommendations for future work that may merit further investigation.

2. *Drug Substance Manufacturing at Amgen and Relevant Details of Production Reactor Filling at Amgen Rhode Island*

As described in the introduction to this document, the biopharmaceutical industry delivers therapeutic proteins to patients. Amgen participates in a broad spectrum of activities related to the delivery of these therapeutics, which includes: (1) drug discovery and development, (2) drug substance (active ingredient) and drug product (finished good that is administrable to patients) manufacturing, and (3) sales and marketing of drug product. Amgen operates a number of facilities, which are dedicated to one or more of these activities, across the world including a network of drug substance manufacturing facilities [4]. The internship upon which the content of this document is based was performed at the Amgen Rhode Island (ARI) drug substance manufacturing facility. This chapter contains a description of the impact that an improvement made at ARI would have on the network.

As its product portfolio evolves over time, Amgen must periodically decide where it will manufacture its commercial and clinical drug substance. Some available production sourcing options include its existing manufacturing facilities and construction of new plants. Efficiency improvements that enable the manufacture of additional material in a given amount of time, such as optimization enabled by the improvement described in this report, liberate time in existing facilities that may be used to accommodate additional production. Liberation of production time in facilities that are capable of producing multiple products, such as ARI [5,6], provides flexibility for the drug substance network as a whole. This flexibility is valuable as it may enable the costs of expanding manufacturing capacity, building a new plant, for example, to be avoided or delayed.

Drug substance production involves a number of activities, which include cell culture scale-up, protein production, and purification [7]. This document is focused on a particular filling operation that takes place during the protein production step. Additional description of the other activities taking place during drug substance manufacturing is therefore outside the scope of this report. Information related to other aspects of production at ARI is provided in [8] and [9].

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[4] "Amgen Manufacturing Fact Sheet."
[5] Pasanek, "The Conclusion of a Biologic's Lifecycle."
[6] Kolata, "Rare Mutation Ignites Race for Cholesterol Drug - NYTimes.com."
[7] Shuler and Kargı, *Bioprocess Engineering / Michael Shuler, Fikret Kargi.*
[8] Pasanek, "The Conclusion of a Biologic's Lifecycle."
[9] Donohue, "Application of Queueing Theory in Bulk Biotech Manufacturing."

As noted above, this document is focused on a particular cell culture filling operation. This operation involves the 20,000 L Production Reactors [10] installed at ARI in which therapeutic protein is expressed. Prior to the start of the fill operation, the Production Reactor contains a quantity of liquid. At the start of the fill operation, flow of liquid from a source tank into the Production Reactor is initiated. During the fill, the source tank liquid, which contains the CCP species, is continuously mixed with the contents of the Production Reactor. After the fill is completed, a homogeneous, post-fill CCP concentration is reached in the Production Reactor. Because the CCP concentration of the source tank liquid is greater than the post-fill CCP concentration in the Production Reactor, the source tank material is hereafter referred to as concentrate.

The Production Reactors are equipped with a number of instrumentation and sampling ports. The CCP concentration probe is installed in one of these ports. The reactors are also equipped with turbine agitation, gas sparging, and a control system to maintain system setpoints, such as that for temperature. Turbine agitation is used to facilitate mixing of the cell culture during the protein production step. While gas sparging also contributes to the agitation of the system, it is used to control certain aspects of the chemistry of the culture suspension and may not be used for mixing control. During the filling operation of interest, the following Production Reactor parameters are nominally identical between batches: (1) culture suspension composition and temperature, (2) turbine agitation and baseline sparge gas flow, and (3) volume in the Production Reactor at the start of the fill. The total sparge flow varies to some extent between batches as the control system adjusts the sparge to control chemistry. This aspect of the fill process is discussed in additional detail in Section 5.3.4. Additional detail regarding parameters, such as reactor, impeller, and sparger geometry, fluid properties, and process setpoints, is proprietary and is not included in this document.

3. *LITERATURE REVIEW*

The questions central to the work described in this document are: (1) what is the variability of the performance of concentration-based control?, and (2) what economic benefit may be attained as a result of process optimization enabled by this performance improvement?

[10] Ibid.

3.1. Scope of Literature Review

For the reasons discussed in Section 1.4, namely considerations of cost and schedule, the variability of the performance of concentration-based control is addressed using a simulation approach. A Monte Carlo framework is used to simulate: (1) the transient CCP concentration profile in the Production Reactor when material is injected, and (2) real-time measurement of the CCP concentration during the fill. A literature review is needed to identify a suitable means of numerically simulating the transient CCP concentration profile during the fill. This review is summarized in Section 3.2.

As described in Section 1.2, developing business cases to support the implementation of new technologies in an existing biopharmaceutical manufacturing facility was the goal of the internship upon which the content of this document is based. The Newsvendor framework is a well-known concept in operations management, see [11] and [12], for example. In the context of the business case developed for concentration-based control, this framework provides an acceptably detailed means of quantifying the economic benefit enabled by this process improvement. Adaptation of the Newsvendor framework to the problem of interest is described in Sections 7.1 and 7.2. Given the flexibility of this framework and its fitness for purpose, potential alternative means of assessing the cost of variability in the context of the problem of interest are not discussed in this document.

3.2. Transient Concentration Profiles during Agitated Tank Batch Filling Operations

Given the engineering-probabilistic simulation approach chosen for use in this work, a means of simulating the transient CCP concentration profile in the Production Reactor during a fill is needed. In the limit of an ideal stirred tank reactor, a volume of material injected into the tank is assumed to reach instantaneous homogeneity with the tank's contents [13]. When this idealized model provides an acceptable degree of accuracy, the transient CCP concentration profile during the fill may be derived using a straightforward species balance. For cases in which the timescale of homogenization is significantly shorter than that of the process of interest, a first

[11] McClain and Thomas, *Operations Management : Production of Goods and Services / John O. McClain, L. Joseph Thomas.*

[12] Cachon and Terwiesch, *Matching Supply with Demand : An Introduction to Operations Management / Gérard Cachon, Christian Terwiesch.*

[13] Schmidt, *The Engineering of Chemical Reactions / Lanny D. Schmidt.*

order chemical reaction whose kinetics are sufficiently slower than the mixing process, for example, the assumption of instantaneous mixing may provide acceptable accuracy. As discussed in Section 5.2, the idealized stirred tank model is inadequate under the conditions relevant to the Production Reactor filling operation of interest.

Homogenization of material injected into an agitated tank occurs via a number of physical processes including convection due to bulk circulation of the injected material induced by agitation of the tank [14,15] and dispersion of the material due to turbulent eddy transport [16,17]. The flow and dispersion induced by these processes lead to both spatial and temporal variations in concentration during a tank batch filling operation [18,19,20]. Several aspects of the tank-liquid-gas system, such as tank geometry, agitation intensity, gas flow rate, and fluid properties, are known to affect the liquid velocity profile that forms within the tank and thus the mixing characteristics of the reactor [21,22,23,24].

No methodologies for modeling the transient concentration profile of a species during a sustained injection of material into an agitated tank were identified through this literature review. Treatment of the injection of a pulse of tracer material, often an inert chemical species, into a stirred tank, however, is well established and modeling of the time required to reach a given degree of homogeneity within the tank after the tracer injection is well documented [25]. The mixing time achieved in an agitated tank refers to the time required for a measured quantity, e.g., solution conductivity, to remain, over time, within a specified fraction of its eventual steady state value. A variety of relations correlating mixing time to factors such as impeller speed, geometry, type, and number, sparge gas flow rate, and vessel geometry are available, for example, [26,27,28,29,30]. While the engineer may find such mixing time correlations useful for reactor

[14] McCabe, Smith, and Harriott, *Unit Operations of Chemical Engineering.*
[15] Ibid.
[16] Kawase and Moo-Young, "Mixing Time in Bioreactors."
[17] Ghanem et al., "Static Mixers."
[18] McCabe, Smith, and Harriott, *Unit Operations of Chemical Engineering.*
[19] Vasconcelos, Alves, and Barata, "Mixing in Gas-Liquid Contactors Agitated by Multiple Turbines."
[20] Hadjiev, Sabiri, and Zanati, "Mixing Time in Bioreactors under Aerated Conditions."
[21] McCabe, Smith, and Harriott, *Unit Operations of Chemical Engineering.*
[22] van't Riet and van der Lans, "Mixing in Bioreactor Vessels."
[23] Hadjiev, Sabiri, and Zanati, "Mixing Time in Bioreactors under Aerated Conditions."
[24] Gogate, Beenackers, and Pandit, "Multiple-Impeller Systems with a Special Emphasis on Bioreactors."
[25] Levenspiel, *Tracer Technology [electronic Resource] : Modeling the Flow of Fluids / Octave Levenspiel.*
[26] McCabe, Smith, and Harriott, *Unit Operations of Chemical Engineering.*
[27] Levenspiel, *Tracer Technology [electronic Resource] : Modeling the Flow of Fluids / Octave Levenspiel.*
[28] Gogate, Beenackers, and Pandit, "Multiple-Impeller Systems with a Special Emphasis on Bioreactors."

design and other purposes, this measure alone does not provide a means of modeling the entire transient concentration profile observed at a particular location within the tank as it mixes. Further complexity is introduced by the fact that the concentration profile may not approach steady state in a monotonic fashion as a result of the circulating nature of the flow induced by the tank agitation.

Three methods that may be used to model the temporal and spatial concentration profiles associated with a batch filling operation in an agitated tank are described in this section: (1) compartment models, (2) a model based on residence time distribution (RTD) theory, and (3) computational fluid dynamics (CFD) models. Using these models, deviations from ideal stirred tank behavior may be addressed. Brief descriptions of each model and its applications, strengths and limitations are provided in the following discussion.

The spatial variation of the mixing characteristics of the vessel may be approximated by segregating the total volume of the vessel into modeled compartments, for example, [31,32,33,34,35]. For example, Mayr et al. provide a compartment representation of a gassed batch reactor with turbine agitation in which the vessel volume is approximated as a series of interconnected ideal stirred tanks [36]. With this formulation, the transient tracer concentration profile in each of the modeled compartments is given by solution of the system of differential equations representing conservation of the tracer as it flows between the compartments and disperses within the bulk fluid. Other idealized flow constructs in addition to the ideal stirred tank, such as ideal plug flow (also known as piston flow), short-circuiting, and stagnant regions, may also be included in a compartment model, for example, [37] and [38]). Using these idealized constructs, compartment models may be formulated with substantial flexibility. Additionally, given the segregation of the vessel volume into conceptually friendly sub-units, compartment models may also offer a more readily understandable physical representation of the complicated

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[29] Vasconcelos, Alves, and Barata, "Mixing in Gas-Liquid Contactors Agitated by Multiple Turbines."

[30] Paul, Atiemo-Obeng, and Kresta, *Handbook of Industrial Mixing : Science and Practice / Edited by Edward L. Paul, Victor A. Atiemo-Obeng, Suzanne M. Kresta.*

[31] Levenspiel, *Tracer Technology [electronic Resource] : Modeling the Flow of Fluids / Octave Levenspiel.*

[32] Mayr et al., "Mixing-Models Applied to Industrial Batch Bioreactors."

[33] Vasconcelos, Alves, and Barata, "Mixing in Gas-Liquid Contactors Agitated by Multiple Turbines."

[34] Magelli et al., "Mixing Time in High Aspect Ratio Vessels Stirred with Multiple Impellers."

[35] Behin and Bahrami, "Modeling an Industrial Dissolved Air Flotation Tank Used for Separating Oil from Wastewater."

[36] Mayr et al., "Mixing-Models Applied to Industrial Batch Bioreactors."

[37] Levenspiel, *Tracer Technology [electronic Resource] : Modeling the Flow of Fluids / Octave Levenspiel.*

[38] Van de Vusse, "A New Model for the Stirred Tank Reactor."

bulk mixing process. Despite these strengths, introducing complexity comes with costs. First, with increasing complexity, it is often necessary to obtain additional information regarding the characteristics of the system, the actual exchange flow rates between adjacent compartments [39], for example, and/or make additional simplifying assumptions, for example, [40]. Obtaining such information requires the ability to measure quantities, such as flow characteristics, at multiple locations within the vessel. Installing additional instrumentation in the ARI Production Reactors, which are commissioned for manufacturing of cGMP material (i.e., material manufactured in accordance with standards and practices enforced by the United States Food and Drug Administration), for the purpose of testing is not tractable. Second, by definition, the idealized models used to formulate a compartment model provide an imperfect representation of the actual physical process of mixing taking place in the vessel. Due to the difficulty in validating the appropriateness of these idealized assumptions when appropriate data are not available, it is desirable to limit the number of idealized assumptions made in modeling the performance of the reactor.

The modeling method based on RTD theory enables the simulation of the transient concentration profile at a given location within the vessel [41]. The RTD framework provides a means of describing the flow behavior that develops in non-ideal vessels [42]. In the case of a continuous flow reactor, the RTD describes the probability that a molecule entering the vessel will reside in the vessel for a given amount of time [43]. As the RTD is a probability distribution, the mean and variance, which provide measures of central tendency and spread, respectively, are often of interest. While there is no flow of material exiting a batch reactor, the RTD concept applies if one considers the probability distribution describing the first time in which a molecule passes a particular location in the vessel, the location of a measurement probe, for example. Such a distribution is referred to as a single-pass RTD [44]. Khang and Levenspiel propose a methodology, referred to hereafter as the KL Model, based on the single-pass RTD determined

[39] Vasconcelos, Alves, and Barata, "Mixing in Gas-Liquid Contactors Agitated by Multiple Turbines."
[40] Van de Vusse, "A New Model for the Stirred Tank Reactor."
[41] Khang and Levenspiel, "New Scale-up and Design Method for Stirrer Agitated Batch Mixing Vessels."
[42] Schmidt, *The Engineering of Chemical Reactions / Lanny D. Schmidt.*
[43] Ibid.
[44] Khang and Levenspiel, "New Scale-up and Design Method for Stirrer Agitated Batch Mixing Vessels."

for the vessel by which the transient concentration profile of a tracer species may be modeled [45]. This model applies for any RTD with small variance and is given as follows [46]:

$$y(t) \cong 1 + 2e^{-\frac{2\pi^2\sigma_\theta^2}{T}t}cos\left(\frac{2\pi}{T}t + 2\pi\sigma_\theta^2\right) \quad (1)$$

where y is the dimensionless impulse response of a recycle stream, which in the case of interest represents the transient dimensionless concentration profile of an injected tracer as it mixes, t is time, σ_θ^2 is the dimensionless variance of the single-pass RTD, and T is the mean of the single-pass RTD. The dimensionless variance is defined as follows [47]:

$$\sigma_\theta^2 = \frac{\sigma^2}{T^2} \quad (2)$$

where σ^2 is the dimensional variance of the single-pass RTD.

This methodology is attractive as it does not require the use of idealized assumptions and requires only two fit parameters, where a compartment model may require more than two parameters depending on its level of sophistication. Additionally, given its relative simplicity, of the three methodologies described in this section this model is the most readily incorporated into a Monte Carlo framework.

CFD modeling provides a third means of understanding the flow patterns that develop within an agitated vessel, for example, [48,49,50,51]. In the context of this document, CFD is considered to be the most flexible and powerful means of modeling a tracer pulse injection described in this section as it can be used to model the concentration profile at any point in an agitated vessel under any reasonable set of operating conditions, such as vessel and agitator geometry, fluid properties, and sparging conditions. Additionally, using CFD, it is possible to investigate both transient and steady state conditions. Finally, CFD simulation does not inherently require the use of idealized assumptions required by compartment models or the lumped approach of an RTD model. These strengths again come with several costs. In the context of the work described in this document, the significant computation time associated with executing complex CFD simulations is the most important drawback of this approach.

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[45] Ibid.
[46] Ibid.
[47] Ibid.
[48] Liu, "Age Distribution and the Degree of Mixing in Continuous Flow Stirred Tank Reactors."
[49] Basheer and Subramaniam, "Hydrodynamics, Mixing and Selectivity in a Partitioned Bubble Column."
[50] Zadghaffari, Moghaddas, and Revstedt, "Large-Eddy Simulation of Turbulent Flow in a Stirred Tank Driven by a Rushton Turbine."
[51] Liew, Nandong, and Samyudia, "Multi-Scale Models for the Optimization of Batch Bioreactors."

Specifically, the long computation time associated with CFD modeling makes this method less amenable for use in generating a statistically robust data set. Second, establishing a representative simulation model of the vessel can be time consuming and costly. Third, while modeling a pulse injection using CFD has been documented in the literature, for example, [52], it may not be practical to model a sustained injection using CFD.

3.3. Modeling Approaches Chosen for Use

The discussion presented in this chapter is intended to provide an analysis and brief summary of published work that is relevant to answering the following questions: (1) how may the transient CCP concentration profile during the fill be numerically simulated?, and (2) what economic benefit may be expected as a result of this performance improvement? Based on this review, the following methodologies are selected for use in the work described in this report:

- The KL Model is selected for use as the foundation of the transient CCP concentration profile model. The transient profile model is described further in Section 5.2.
- As described in Section 3.1, the well-known Monte Carlo and Newsvendor approaches are chosen for use in simulating the performance of the concentration-based control strategy and evaluating the economics of the improvement, respectively.

4. Current State of Production Reactor Fill Operation

In the current state, the Production Reactor fill operation described in this report is controlled using a volume-transfer-based strategy. Just prior to the fill, the volume of concentrate required to achieve the pre-defined target CCP concentration in the Production Reactor is calculated using the following real-time data: (1) the CCP concentration measured in the tank containing the concentrate, and (2) the volume of liquid present in the Production Reactor. The sum of the liquid volume in the Production Reactor and the target concentrate volume constitutes the target final volume immediately after completion of the fill. Concentrate transfer from the source vessel to the Production Reactor is secured when the target final volume is reached in the Production Reactor. The CCP concentration in the Production Reactor is then measured.

[52] Zadghaffari, Moghaddas, and Revstedt, "Large-Eddy Simulation of Turbulent Flow in a Stirred Tank Driven by a Rushton Turbine."

The purpose of this chapter is to provide a characterization of the current state of the performance of this Production Reactor filling operation, which serves as the basis for comparison with the predicted performance of concentration-based control. Additionally, a sample relation between the performance of the fill operation and the overall performance of the protein production step is included for use in the example quantification of the economic impact of this improvement discussed in Chapter 7.

4.1. Data Used to Assess Current State

A number of data are logged for each production run and are subsequently stored in a centralized database. The quantities relevant to the current state assessment documented in this chapter include the: (1) CCP concentration measured in the Production Reactor after completion of the fill (post-fill CCP concentration), and (2) final protein concentration measured at the completion of the protein production step. Data from a representative range of historical production runs are used to characterize the current state of the fill operation. These data are proprietary to Amgen and are thus withheld from this document.

4.2. Characterization of Process Variance

The fractional deviation from the target post-fill CCP concentration from a representative sample of production runs is presented in Figure 1. The fractional deviation provides a means of discussing the variability of the current state without disclosing proprietary data. The following observations are drawn from this plot. First, in the current state, the realized post-fill CCP concentration consistently exceeds the target. Second, the magnitude and variability of the overshoot do not vary much over time. This observation is reasonable as the individual variabilities mentioned above remain constant during the period of observation. The overshoot is considered to be a manageable characteristic of the fill operation, because its average magnitude is consistent over time. The variability of the process introduces a finite probability that a given fill will result in an overshoot that exceeds the pre-defined upper limit post-fill CCP concentration as a result of inherent randomness. Reducing this variability is therefore desirable.

The variability of the performance of the fill operation is introduced by the following: (1) the variability of the concentrate CCP concentration and initial Production Reactor volume measurements, which are made before the start of the fill operation, (2) the variability of the

actual volume of concentrate transferred relative to the target transfer volume, and (3) the variability of the Production Reactor post-fill CCP concentration measurement. A disaggregation of the individual contributions of each of these sources of variability is not required to achieve the purpose of the analysis documented herein and thus is not included in this document. It is noted that the variability introduced by the measurement of the concentration of the CCP concentrate and initial Production Reactor volume would not affect the performance of concentration-based control, because this information would no longer be used to make process decisions. Additional detail regarding the contributions to the variability of concentration-based control is provided in Section 6.2.1.

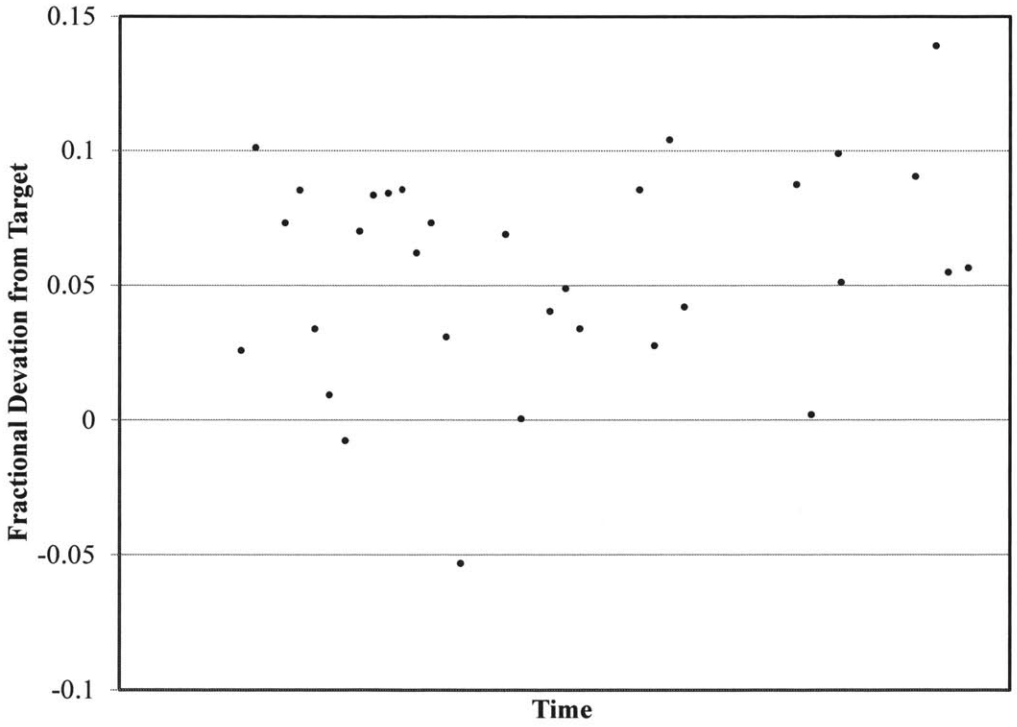

Figure 1 Fractional Deviation from Target Post-Fill CCP Concentration from Production Runs Representative of the Current State

In order to evaluate the impact of concentration-based control on the performance of the fill operation, it is necessary to characterize the variability of the overshoot described above. On average, the current state fill process leads to an overshoot of approximately 6% of the post-fill target and a standard deviation in post-fill CCP concentration equal to approximately 4% of the target. It is noted that because the variability of the current state may be affected by other process changes, when evaluating concentration-based control for potential implementation, it would be

necessary to update the current state performance evaluation. Based on a Shapiro-Wilk W Test, a normal distribution provides an adequate description of the post-fill CCP concentration data. A normal distribution is therefore assumed for the purpose of making statistical inferences related to the current state elsewhere in this document.

4.3. Relationship between Post-Fill CCP Concentration and Process Yield

Reducing the variability of the fill operation is beneficial for a variety of reasons including opportunities for process optimization enabled by tightened process control. For molecules in which the protein titer contained in the culture broth at the completion of the protein production step is positively correlated with the post-fill CCP concentration, batch-wise product yield may be increased by increasing the post-fill CCP concentration realized after each fill. Since the quantitative relationship between final titer and post-fill CCP concentration for a given molecule is proprietary to Amgen, such information is not included in this document.

For the purpose of demonstrating the framework developed to quantify the benefit of reducing the variability of the fill operation, the following relationship between final titer and post-fill CCP concentration is applied in the analysis detailed in Chapter 7.

$$M_P = Y \cdot CCP_{PF} + constant \quad (3)$$

where M_P is the mass of protein produced during a batch, Y is the production step yield, and CCP_{PF} is the measured post-fill CCP concentration. The following points are made regarding the above relation. First, this form is not intended to represent the fundamental processes taking place during protein expression. It is chosen for its simplicity in the demonstration of the optimization framework described in Section 7.2. Second, the form of the above relation may or may not represent the true behavior of the Amgen production process during which the fill operation occurs.

5. Modeling of Production Reactor CCP Concentration during Fill Operation

An overview of the current state of the fill operation is provided in the introduction to Chapter 4. In that overview, the current, volume-transfer-based control strategy is described. Recall that under concentration-based control, the Production Reactor fill operation would be controlled using the inline CCP concentration measurement rather than the volume measurement.

Specifically, under this process control strategy, the flow of concentrate into the Production Reactor would be secured after a pre-defined, measured CCP concentration in the Production Reactor is reached.

As described in the introduction to Chapter 3, a simulation approach is used to predict the performance of concentration-based control. A means of modeling the transient CCP concentration profile in the Production Reactor during a fill is therefore needed to enable the prediction of the performance of concentration-based control using a simulation approach. The model used to simulate the transient CCP concentration profile in the Production Reactor at the location of the measurement probe is developed in this chapter.

5.1. Data Used to Determine Transient Concentration Profile Model Parameters

The concentrate source vessels and Production Reactors are outfitted with a number of instrumentation probes that provide continuous monitoring of various process parameters. The time series of data recorded by each of these instruments are archived in a centralized database. The following data recorded during a number of historical production runs are used to determine the model parameters described in Section 5.2: (1) Production Reactor CCP concentration, (2) Production Reactor volume, and (3) concentrate source vessel CCP concentration. These data are proprietary to Amgen and are thus withheld from this document. Selected results of the analysis described in this chapter are presented in Appendix B .

The instruments used to measure these data are described as follows. The raw signal measured by the CCP concentration probe is translated to CCP concentration using proprietary correlations relating probe signal to concentration measured using an offline assay. Additional detail regarding the probe and offline assay is proprietary and is withheld from this document. A correlation developed specifically for the source vessel is used to quantify source CCP concentration and a correlation developed specifically for the Production Reactor culture is used to quantify production culture CCP concentration. Due to the proprietary nature of these correlations, they are not included in this document. The Production Reactor volume is quantified using a mass measurement instrument, whose signal is converted to volume.

To limit the size of the data set stored by the production server, the raw signals measured by the various process instruments are retained on the server at varying frequencies. These

frequencies are determined using a combination of inputs from plant personnel and the difference between the current measured value provided by the instrument and the last several values archived by the system. As a result, the data recorded for each of the instruments described above are not necessarily available at the same points in time. Since the Production Reactor CCP concentration is the measured quantity to which the mixing model parameters are fit, raw CCP concentration probe readings and associated measurement times are retrieved from the server. These data are available at an approximate frequency of 1% of the mean of the single-pass RTD. The time series associated with the Production Reactor CCP concentration raw data is used to define the times at which the values of the other parameters are retrieved from the server. Specifically, the values for the source CCP concentration and the Production Reactor volume used in this analysis are interpolated from the raw values maintained on the server at each of the times at which a Production Reactor CCP concentration measurement was recorded. The use of these interpolated values is a limitation of the available data set. This limitation is considered acceptable, because neither the real-time source CCP concentration nor the real-time Production Reactor volume measured during the fill is directly used to determine the model fit parameters. This aspect of the model fitting approach is described further in Section 5.3.

5.2. Transient Concentration Profile Model Development

As shown in Figure 7, the measured CCP concentration in the Production Reactor increases by a non-negligible amount after the flow of concentrate into the Production Reactor is secured. This observation indicates that assuming ideal mixing is inadequate for modeling the transient CCP concentration profile.

A publicly available methodology for modeling the transient concentration profile of a species during a sustained injection of material into an agitated tank was not identified through the literature review described in Section 3.2, but methods are available to model the transient concentration profile of a pulse of a tracer material injected into a batch stirred tank. For the reasons described in Section 3.2, the KL Model, which is reproduced as Equation (1), is selected for use as the foundation of the model used to simulate the transient CCP concentration profile during the fill.

Because the KL Model is derived for use in describing the transient response of an agitated batch vessel following the injection of a tracer, it is necessary to adapt this model for use

in describing the transient behavior associated with a sustained batch injection operation, the physical process of interest in this report. The first step in adapting the KL Model for the intended use is to express it in dimensional form in terms of the variables of interest:

$$X(t) = \frac{(\Delta V \cdot X_S)}{V(t)}\left[1 + 2e^{-\frac{2\pi^2\sigma_\theta^2}{T}t}cos\left(\frac{2\pi}{T}t + 2\pi\sigma_\theta^2\right)\right] \quad (4)$$

where X is the modeled CCP concentration in the Production Reactor, t is time, V is the volume in the Production Reactor, ΔV is the volume of concentrate entering the Production Reactor, X_S is the source CCP concentration (i.e., the CCP concentration of the concentrate), and the variables contained in the bracketed term are as defined in the explanation of Equation (1). For readability, the definitions of these variables are restated as follows: σ_θ^2 is the dimensionless variance of the single-pass RTD and T is the mean of the single-pass RTD. It is noted that Equation (4) applies when the time required to inject ΔV is significantly less than the mean of the single-pass RTD. Conformance with this requirement is confirmed at the end of this section.

It is evident that the first term on the right-hand side (RHS) of Equation (4) represents the steady state CCP concentration after the Production Reactor has mixed to homogeneity (i.e., it is the mass of material entering the Production Reactor resulting from the addition of a finite volume of concentrate divided by the total volume of liquid in the vessel). The bracketed term on the RHS of Equation (4) provides a means of modeling the transient response of the system as it approaches steady state. It also enables the magnitude of the deviation of the transient concentration from steady state to be modeled using an exponential decay, which is the expected behavior of the system [53]. The system response model exhibits appropriate long-time behavior as the bracketed term approaches unity at long times, thus, the appropriate steady state concentration is given by Equation (4) after a sufficiently long mixing period. Equation (4) provides a poorer description of the system at times close to zero. For example, for certain values of the single-pass RTD parameters, the transient CCP concentration modeled using Equation (4) is less than zero, which is nonphysical. Despite this limitation of the system response model, when successive responses are offset from one another and summed together, the aggregate response curve is smooth. The Production Reactor fill operation requires a sufficiently long period of time that this dampening effect is observed. The modeled system response to a series of sequentially injected pulses is shown in Figure 2 to demonstrate this aspect of the model. As

[53] Khang and Levenspiel, "New Scale-up and Design Method for Stirrer Agitated Batch Mixing Vessels."

shown in Figure 2, the response to a series of pulses is smooth until the flow of concentrate into the vessel is secured, which is consistent with the behavior of the real system (see Figure 7). Thus, the poorer short-time performance of Equation **(4)** is acceptable given the intended use of this model.

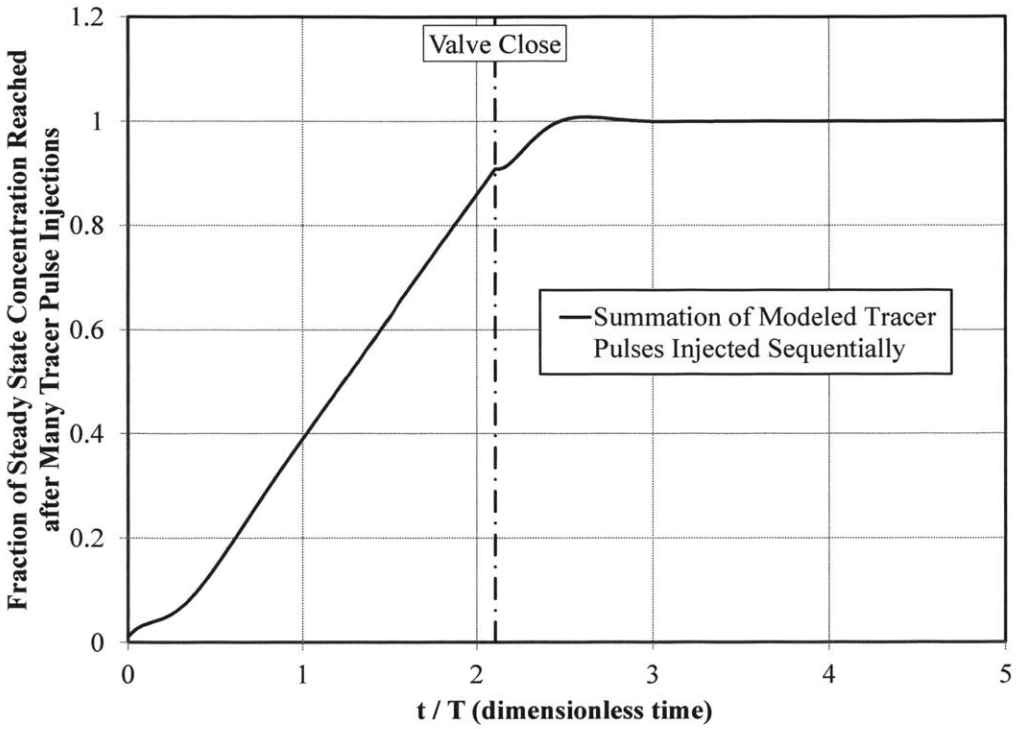

Figure 2 **Example Modeled System Response to Series of Sequentially Injected Tracer Pulses**

The next step in adapting the KL Model for use in a sustained injection of liquid into a batch stirred tank is to discretize the entirety of the filling operation into a series of successive "pulse injections" and summing the transient responses given by Equation **(4)** for each of these pulses. This step is stated mathematically:

$$X(t) = \sum_i \frac{(\Delta V \cdot X_S)_i}{V(t)} \left[1 + 2e^{-\frac{2\pi^2\sigma_\theta^2}{T}(t-t_i)} cos\left(\frac{2\pi}{T}(t-t_i) + 2\pi\sigma_\theta^2\right)\right] \qquad \textbf{(5)}$$

where the subscript, i, refers to the i^{th} Production Reactor CCP concentration measurement recorded after the start of the fill operation and the other variables are as previously defined. The physical meanings of the individual terms are described above. Equation **(5)** simply represents the summation of a number of pulse injections flowing into the Production Reactor in succession, which in aggregate, provides a representation of the CCP concentration in the

Production Reactor as a function of time during a filling operation. The model given by Equation (5), and subsequent derived versions of Equation (5), is hereafter referred to as the SKL Model (Summed KL Model).

The decay constant, K, used by Khang and Levenspiel [54] is introduced to simplify Equation (5):

$$X(t) = \sum_i \frac{(\Delta V \cdot X_S)_i}{V(t)} \left[1 + 2e^{-K(t-t_i)} cos\left(\frac{2\pi}{T}(t - t_i) + 2\pi\sigma_\theta^2\right)\right] \quad (6)$$

$$K = \frac{2\pi^2\sigma_\theta^2}{T} \quad (7)$$

As described in Section 5.1, the frequency of availability of the CCP concentration probe measurement data is approximately 1% of the mean of the single-pass RTD. This "pulse" duration as a fraction of the mean of the single-pass RTD is comparable to that achieved in the experiments against which the KL Model was originally validated [55]. It is therefore considered reasonable to use Equation (1) as the basis for the transient response of the system. That is, the "pulse" duration is significantly less than the mean of the single-pass RTD, meaning that it is reasonable to approximate each "discrete" volume injection as a pulse.

5.3. Transient Concentration Profile Model Fitting Approach

The adjustable parameters present in Equation (5), the mean, T, and dimensionless variance, σ_θ^2, of the single-pass RTD, are determined for each of a number of historical executions of the fill operation by fitting the model given in Equation (5) to historical data. The fit is performed using the Microsoft Excel Solver tool to minimize the sum of the squared differences between the modeled CCP concentration and the measured CCP concentration. This software is selected for use for this task, because it is readily available for use by Amgen personnel.

To perform the fit, it is assumed that the entirety of the volume of material injected into the Production Reactor during a given interval enters at the start of the interval. For example, during the interval defined by the start of the fill and the first CCP concentration measurement made during the fill (i.e., the first injected pulse), it is assumed that the volume entering the

[54] Ibid.
[55] Ibid.

Production Reactor during this interval enters instantaneously at the time of the start of the fill. This approach is consistent with the theoretical construct of an ideal pulse injected into the vessel at the start of a measurement interval.

To facilitate fitting Equation (6) to historical data, it is convenient to factor the continuous time, t, out of the summation:

$$X(t) = \frac{1}{V(t)} \left[\begin{array}{c} \sum_i (\Delta V \cdot X_S)_i + \\ 2e^{-Kt} \cdot cos\left(\frac{2\pi}{T} t\right) \sum_i (\Delta V \cdot X_S)_i \cdot e^{Kt_i} \cdot cos\left(2\pi\left(\sigma_\theta^2 - \frac{t_i}{T}\right)\right) - \\ 2e^{-Kt} \cdot sin\left(\frac{2\pi}{T} t\right) \sum_i (\Delta V \cdot X_S)_i \cdot e^{Kt_i} \cdot sin\left(2\pi\left(\sigma_\theta^2 - \frac{t_i}{T}\right)\right) \end{array} \right] \quad (8)$$

The derivation of Equation (8) is provided in Appendix A .

Due to the nature of the available data, it is necessary to make a number of decisions related to specific aspects of the fitting approach. The rationale for each of these decisions is described in Sections 5.3.1 through 5.3.4. Per the discussion given in Sections 5.4 and 6.2.2, the results of the transient concentration profile modeling are considered to be reasonable, which supports the reasonableness of the model fitting decisions described in Sections 5.3.1 through 5.3.4.

5.3.1. Production Reactor Volume Profile

Due to variability in the measured value of the Production Reactor volume during the fill, the variables V and ΔV of Equation (5) are modeled under the assumption that the flow rate of concentrate from the source vessel into the Production Reactor is constant. Specifically, when the flow rate of concentrate during each interval is calculated as the ratio of the change in volume to the time duration of the interval, the flow rate profile over the duration of the fill is highly variable. A representative flow rate profile determined using this calculation approach is presented in Figure 3. Given that the fill is performed as a fixed-pressure transfer, a steady flow rate would be expected; thus, the variable behavior depicted in Figure 3 is considered to be unrepresentative of the system. Additionally, there is minimal variation about a linear fit applied to the measured volume time series recorded during the fill, which further supports the use of a constant flow rate. A representative plot of such a linear fit is provided in Figure 4. The variables

V and ΔV of Equation **(5)** are thus modeled as follows: (1) V at each time point during the fill is taken as the sum of the initial volume in the Production Reactor and the total volume of concentrate that would have entered the Reactor at a constant flow rate by that time, and (2) ΔV is taken as the product of the flow rate and the time duration of the interval. Finally, the flow rate, itself, is determined as the difference between the measured volumes in the Production Reactor after and before the fill divided by the total time of the fill.

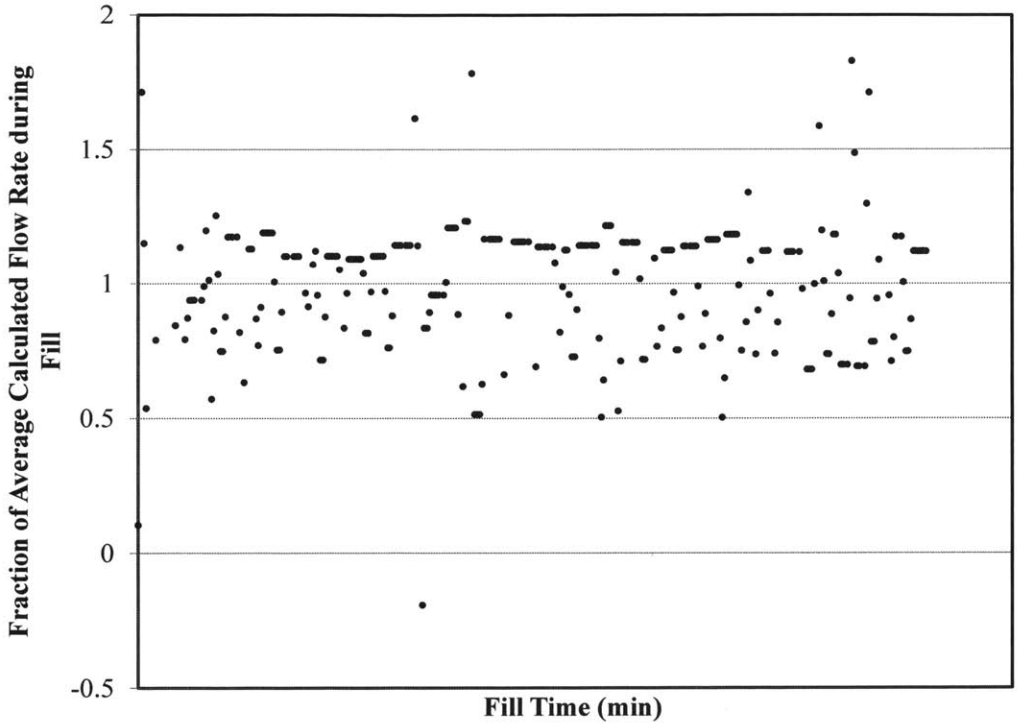

Figure 3 **Concentrate Flow Rate Calculated as the Ratio of the Change in Volume in the Production Reactor to the Time Duration of the Interval**

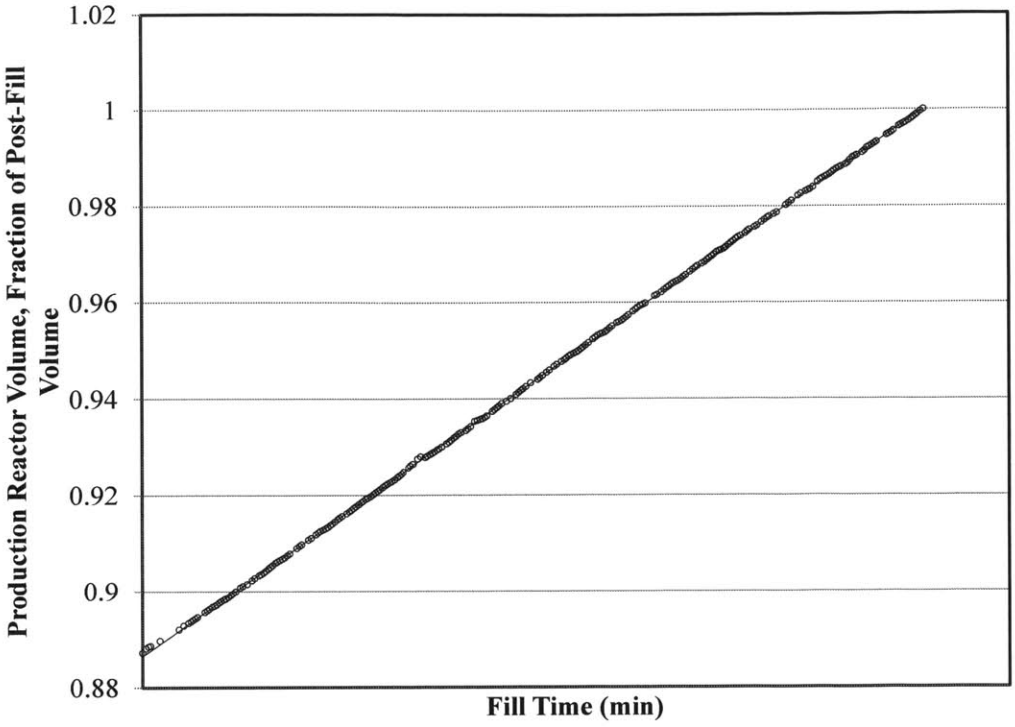

Figure 4 Linear Regression Representation of Concentrate Flow Rate

5.3.2. Source Vessel CCP Concentration

During the fill operation, downward drift in the source vessel CCP concentration measurement is observed. This behavior is not considered to be representative of the true concentration in the source vessel as the concentration would not be expected to vary, because chemical reactions that would cause a decrease in CCP concentration are not expected to be taking place. Thus, the CCP concentration of the liquid in the source vessel during a given fill is taken to be the measured CCP concentration in the source vessel just prior to the start of the fill.

5.3.3. Fill Operation Mass Balance Offset

When fitting the model given by Equation (8) to historical fills, it is observed that there is a discrepancy in the mass balance between the source vessel and the Production Reactor. The discrepancy in the mass balance is demonstrated in Figure 5 for a representative fill. From this figure, it is apparent that the steady state CCP concentration predicted using the mass balance is less than the measured steady state CCP concentration for this particular fill. For this particular fill, the predicted CCP concentration is approximately 2% less than the measured value. As noted

in Section 5.1, the CCP probe signal is converted to units of CCP concentration using proprietary correlations. The mass balance discrepancy is attributed primarily to measurement error introduced by these correlations. This attribution is discussed further in the following paragraph.

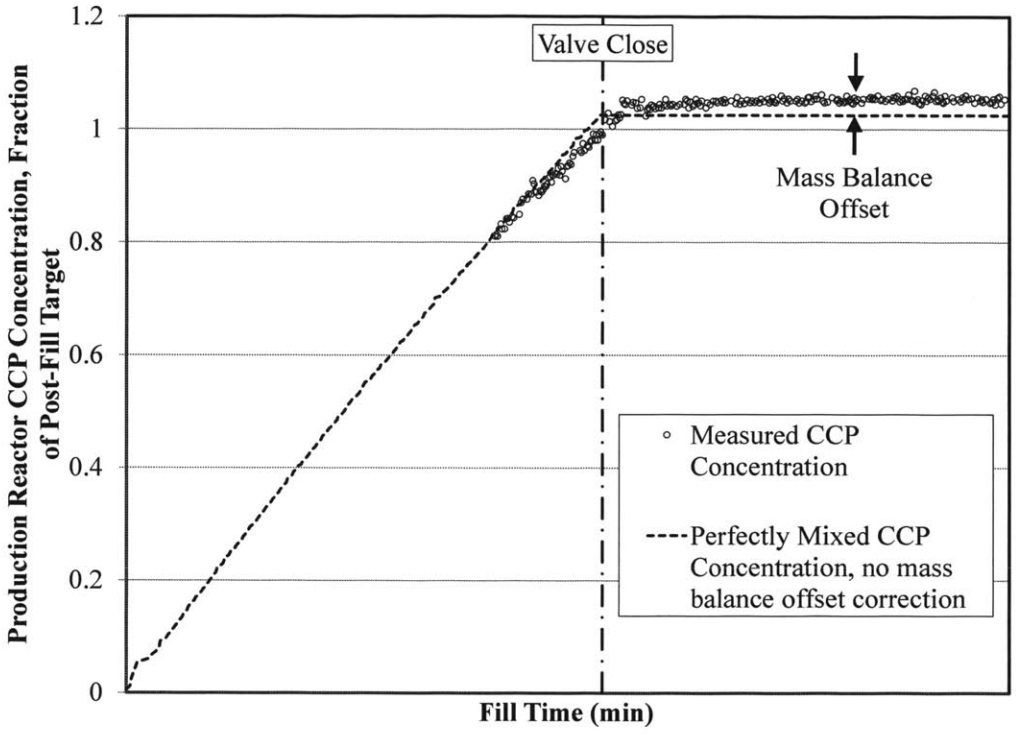

Figure 5 Representative Fill Profile with Modeled Perfectly Mixed CCP Concentration Profile without Mass Balance Offset Correction

For demonstrative purposes, a correlation curve that is qualitatively similar to the proprietary curve for the source vessel is given in Figure 6. Each datum on the plot represents an ordered pair of probe signal and CCP concentration measured using an offline measurement technique applied to a sample drawn from the Production Reactor. As demonstrated by the annotated datum on Figure 6, there is variability about the correlation between probe signal and CCP concentration. Thus, for a fill in which the true CCP concentration of the source vessel is given by the annotated datum, the CCP concentration quantified using the probe (the CCP concentration given by the best fit curve) would be less than the true CCP concentration. In the instance that the Production Reactor CCP measurement is perfectly accurate (i.e., the Production Reactor correlation curve provides the true CCP concentration), the measured steady state CCP concentration in the Production Reactor after the completion of the fill would exceed the calculated steady state CCP concentration based on a mass balance. Said differently, since the

true CCP concentration in the source vessel is greater than the CCP concentration quantified using the probe, a greater-than-expected quantity of CCP species would be transferred, leading to a greater-than-expected Production Reactor CCP concentration. Given that the offset is attributed to random error in the CCP concentration measurements made in the source vessel and Production Reactor, it is expected that this parameter would be zero on average. This expectation is confirmed in Section 5.4.

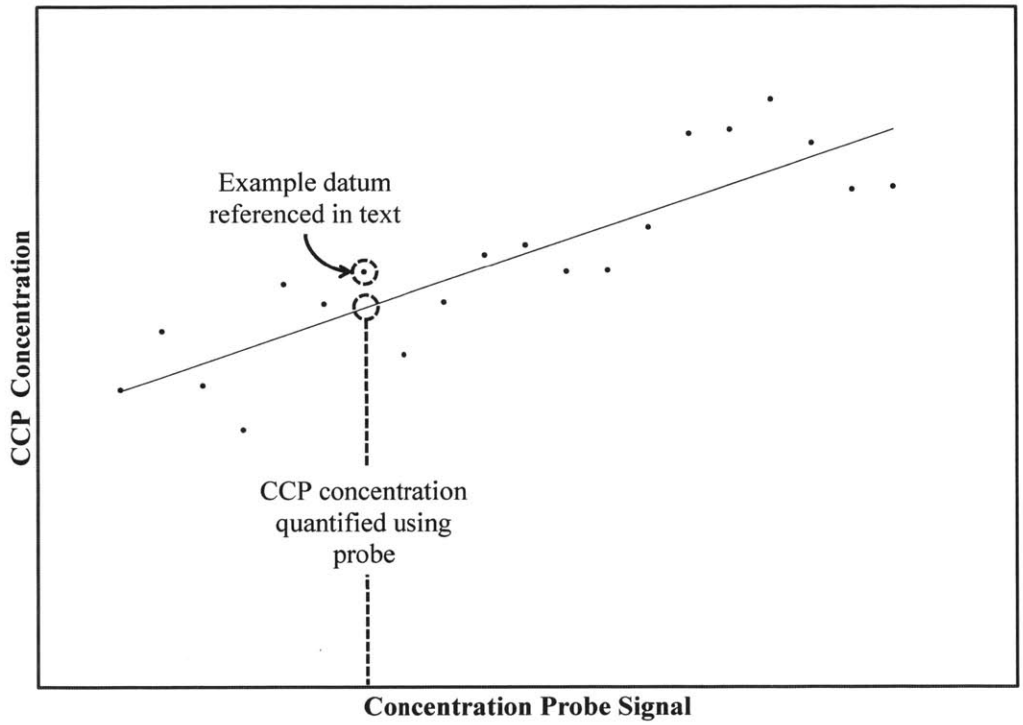

Figure 6 **Qualitative Relation between CCP Probe Signal and Offline CCP Concentration Measurement for Source Vessel (plot data developed using random number generation and are not representative of Amgen performance)**

While the variability causing the mass balance offset is introduced by both the source vessel and Production Reactor correlation curves, it is difficult to justify a particular allocation of the offset to one or both of the curves. The mass balance offset is thus applied entirely to the source vessel CCP concentration measurement.

It is noted that the mass balance offset is not intended for use as a predictive factor as its value may not be determined until after the completion of a given fill; rather, it is used to facilitate fitting Equation **(8)** to historical fill data sets. The CCP concentration of the source vessel is adjusted as follows for the mass balance offset:

$$X_S = X_{S,meas} + F \tag{9}$$

where $X_{S,meas}$ is the CCP concentration in the source vessel measured using the probe and F is the mass balance offset factor determined for the historical fill of interest.

The mass balance offset factor for a given run is derived from a balance on the quantity of CCP species added to the Production Reactor from the source vessel:

$$X_S \Delta V_{tot} = X_{meas} V_f \tag{10}$$

where ΔV_{tot} is the total volume of concentrate added to the Production Reactor, X_{meas} is the steady state CCP concentration in the Production Reactor after the completion of the fill measured using the probe, and V_f is the final volume in the Production Reactor after the completion of the fill. The relation used to calculate the value of the offset factor for each run is then given by combination of Equations (9) and (10):

$$F = \frac{X_{meas} V_f - X_{S,meas} \Delta V_{tot}}{\Delta V_{tot}} \tag{11}$$

5.3.4. Data Included in Model Fit

The rapidity of mixing is of practical importance for concentration-based control. Specifically, when the fill is secured after a pre-defined CCP concentration is measured, the rapidity of mixing affects the extent to which the CCP concentration will rise after the flow of concentrate is stopped. That is, when mixing is rapid, nearly all of the injected concentrate will have mixed to homogeneity at the instant the concentrate inlet valve is closed, leading to a steady state CCP concentration that is slightly greater than the CCP concentration at the time of concentrate inlet valve closure. When mixing is slower, there will be a greater quantity of partially mixed concentrate when the injection is secured, leading to a larger increase in CCP concentration as the vessel mixes to uniformity after valve closure. Given this sensitivity to the rapidity of mixing, it is noted that the gas flow rate into the vessel during a given fill varies by approximately 20%. Gas flow into stirred vessels has been observed to affect mixing characteristics [56,57,58] and it is thus necessary to consider this aspect of the fill in the fitting approach. Note that as a result of the varying gas flow rate during the fill, the values of the mixing parameters determined using the fitting approach described in Section 5.3 represent

[56] Cheng et al., "Experimental Study on Gas–liquid–liquid Macro-Mixing in a Stirred Tank."
[57] Basheer and Subramaniam, "Hydrodynamics, Mixing and Selectivity in a Partitioned Bubble Column."
[58] Gogate, Beenackers, and Pandit, "Multiple-Impeller Systems with a Special Emphasis on Bioreactors."

sparge-averaged values. Also note that this approach is preferable to explicitly accounting for the gas flow rate, using a correlation for gassed mixing, for example, as the sparge is used for culture chemistry control and thus may not be used for mixing control.

A representative fill profile is given in Figure 7. From this figure, it is apparent that the slope of the measured CCP concentration profile decreases somewhat as the completion of the fill is approached. Additionally, the deviation of the CCP concentration profile from the perfectly mixed curve increases near the end of the fill. From these observations, it appears that the mixing process becomes less rapid during this portion of the fill. This apparent slowing down of the mixing process takes place during an increasing trend in gas flow rate, suggesting that the increase in gas flow rate in this operating range leads to a reduction in mixing efficiency. A similar effect (i.e., less efficient mixing with increasing gas flow) has been observed in the literature [59].

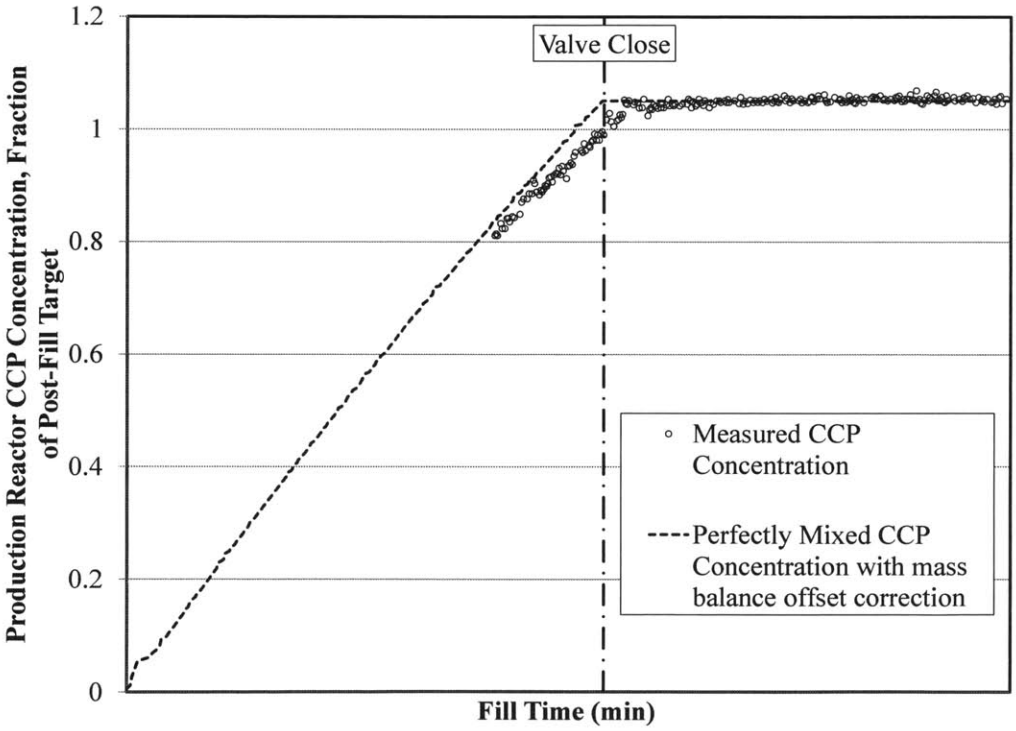

Figure 7 Representative Fill Profile with Modeled Perfectly Mixed CCP Concentration Profile

As described in Section 6.2, it is desirable that the best fit curve provide an accurate fit at the end of the fill. To address the apparent variation in mixing efficiency during the fill,

[59] Ibid.

excluding a portion of the CCP concentration data measured prior to the completion of the fill is evaluated as a means of improving fit accuracy at the end of the fill. In Figure 8, two fits of Equation **(8)** to a representative fill are presented, in which: (1) all measured CCP concentration data are included in the fit, and (2) CCP concentration data measured greater than one minute prior the end of the fill are excluded from the fit. To more clearly display the differences between the two fit curves near the end of the fill, a magnified version of Figure 8 is given as Figure 9. When all CCP data measured before the closure of the concentrate inlet valve are included in the fit, it is evident that the modeled CCP concentration exceeds the measured CCP concentration at the time of completion of the fill. This observation is attributed to the apparent variation in the mixing characteristics of the vessel evidenced by the departure of the CCP profile from the perfectly mixed condition. When the one-minute criterion is applied, the model provides a more accurate fit to the data near the end of the fill. The one-minute criterion is thus applied in the fitting of Equation **(8)** to historical fill data sets.

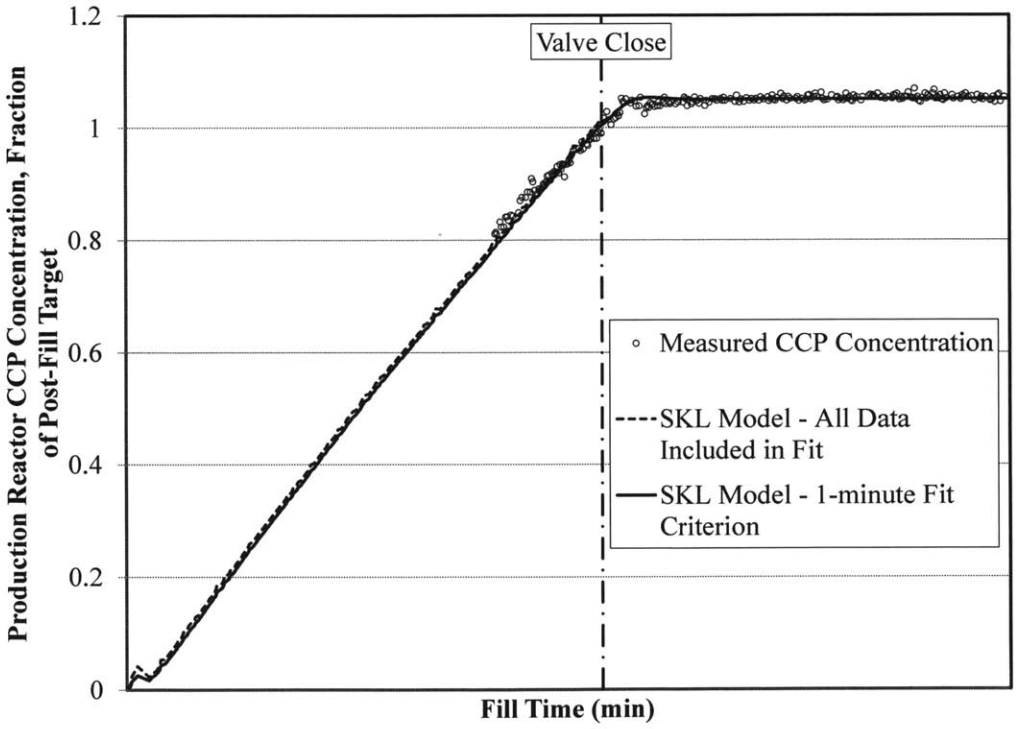

Figure 8 **Representative Fill Profile with SKL Model Fit to: (1) All Measured CCP Concentration Data, and (2) Measured CCP Concentration with One-Minute Criterion Applied**

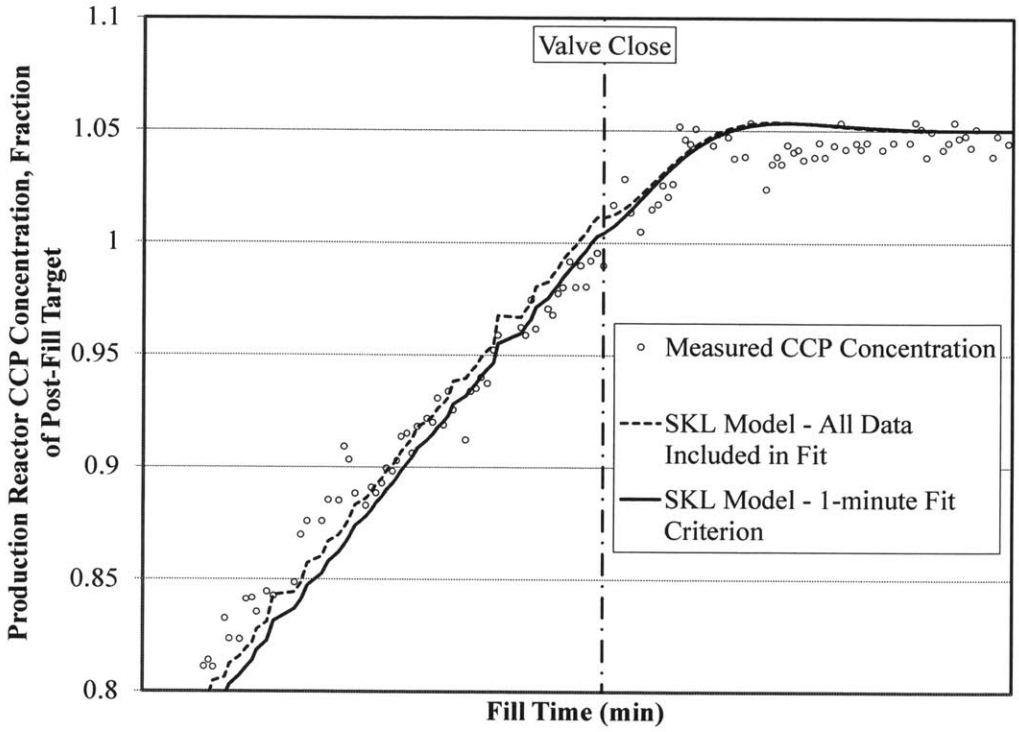

Figure 9 Magnified View of Figure 8

5.4. Results of Transient Concentration Profile Modeling

The model given in Equation **(8)** is fit to a number of historical fill data sets according to the approach described in Section 5.3. Fitting results for each historical data set are provided in Appendix B . Following detailed verification of the calculations used to generate the results presented in this section, it was necessary to make minor changes. It was determined that these changes did not affect the conclusions drawn in this chapter. Numerical values cited in the text were updated as needed to reflect adjustments to the calculations.

The fill profile provided in Figure 8 is reproduced with only the model fitted per Section 5.3 as Figure 10. From Figure 10, it is apparent that the model provides a reasonable fit to the data near the time of closure of the concentrate inlet valve. The appropriateness of the model and fitting approach is further demonstrated by Figure 11 and Figure 12. First, from Figure 11, it is evident that the modeled CCP concentration is in good agreement with the measured CCP concentration during the fill and that the fit is free from obvious bias. Second, from Figure 12, it is evident that the residuals of the fit are centered about approximately zero, suggesting that the fit is accurate over the modeled range. Additionally, the residuals of the fit are normally

distributed, which supports the use of normally distributed concentration measurement variability in the Monte Carlo simulation described in Chapter 6.

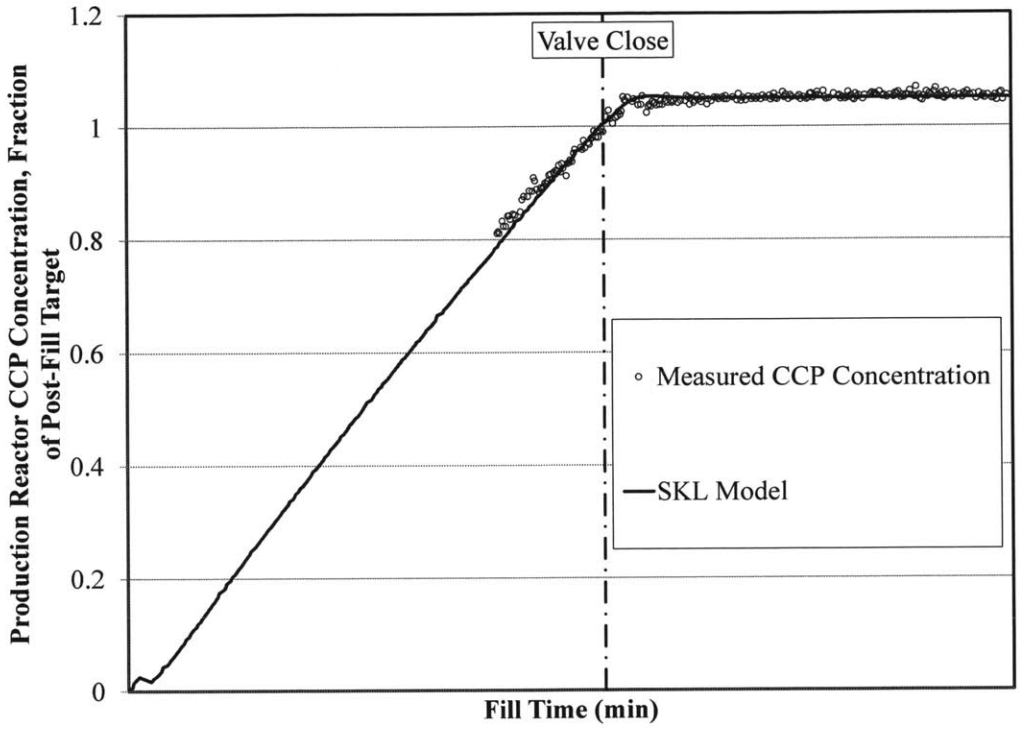

Figure 10 **Representative Fill Profile with Transient Concentration Profile Model Fitted as Described in Section 5.3**

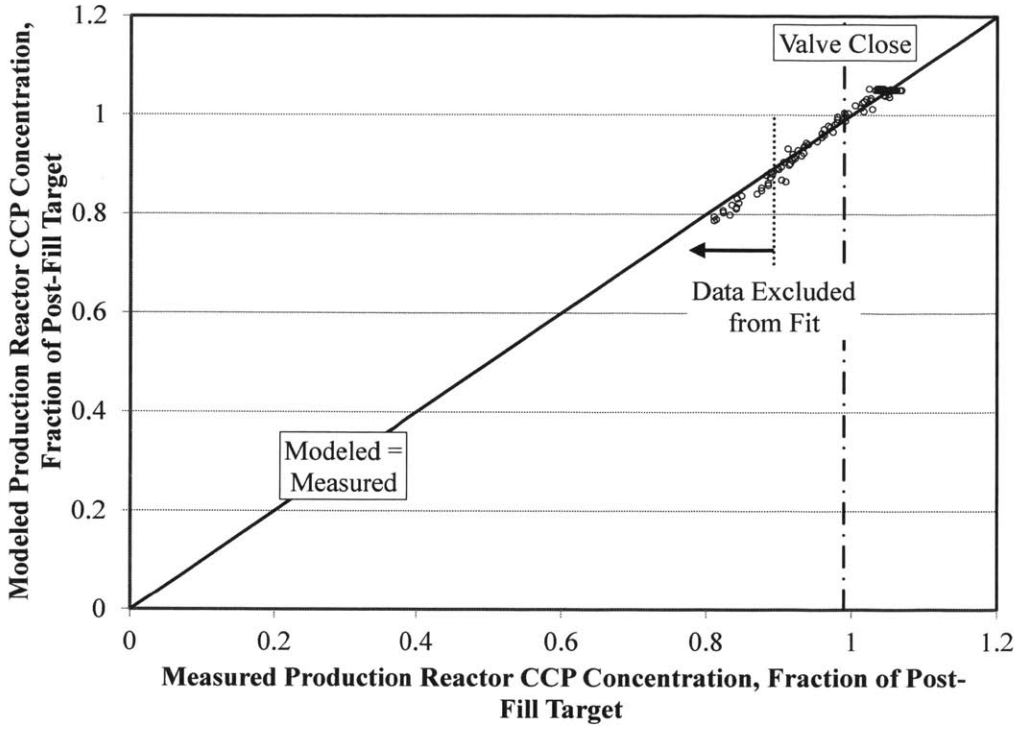

Figure 11 **Modeled CCP Concentration vs. Measured CCP Concentration for a Representative Fill**

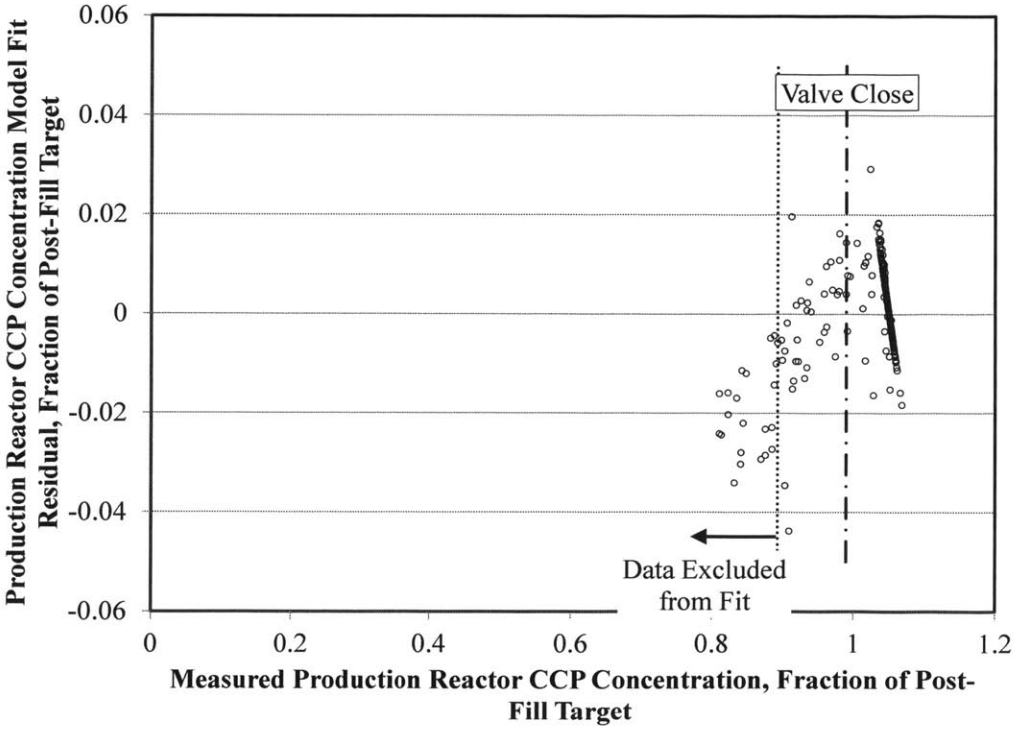

Figure 12 **Modeled CCP Concentration Fit Residual vs. Measured CCP Concentration for a Representative Fill**

The appropriateness of the transient concentration profile model and fitting approach is further evaluated through analysis of the information presented in Appendix B .

First, the physical reasonableness of the RTD fit parameters (the mean and variance, T and σ_θ^2, respectively) is assessed. The response of the Production Reactor to a pulse injection of a tracer is modeled using the mean values of the RTD parameters determined from historical fill data sets and is plotted in Figure 13. Under these conditions, the reactor would mix to within approximately 5% of uniformity after approximately 0.5 time constants. This observation is reasonably consistent with the results of a proprietary Production Reactor tracer study in which uniformity was reached after approximately 0.3 time constants. This level of agreement is considered to be reasonable due to the differences between the conditions of the tracer experiment and those relevant to the culture broth: (1) the tracer experiment was conducted in water rather than cell culture broth, and (2) the average sparge flow rate at the end of the fill for the data sets considered exceeds the flow rate of the tracer experiment by approximately 30%.

Second, potential impacts of the mass balance offset on the fit parameters are evaluated. From Figure 14, it is apparent that there is not a significant correlation between the physical model fit parameters and the mass balance offset correction. This observation suggests that the approach chosen to address the offset does not affect the results of the model fitting. The values of the offset correction determined are bounded within +/-10% of the measured source vessel CCP concentration, which is consistent with the precision of the probe measurements. Finally, it is noted that while on average the offset correction is greater than zero, the difference between the average fractional offset correction and zero is not statistically significant. This result is consistent with the expectation that the offset factor would be zero on average.

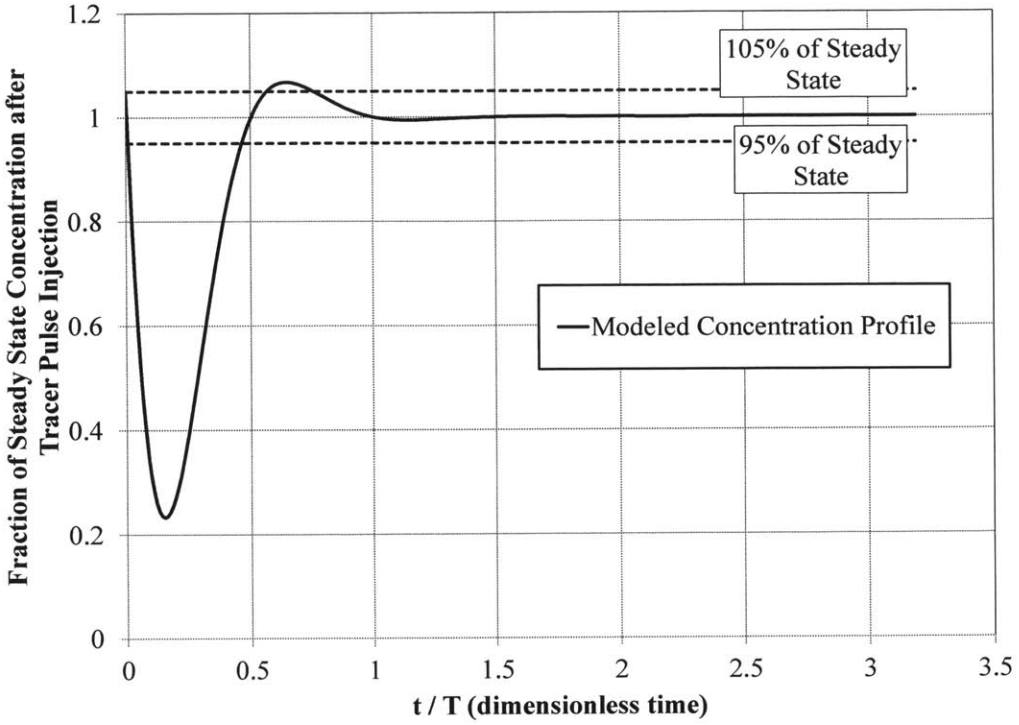

Figure 13 **Production Reactor Transient Response to Tracer Pulse Injection Modeled Using Mean Values of Single-Pass RTD Parameters**

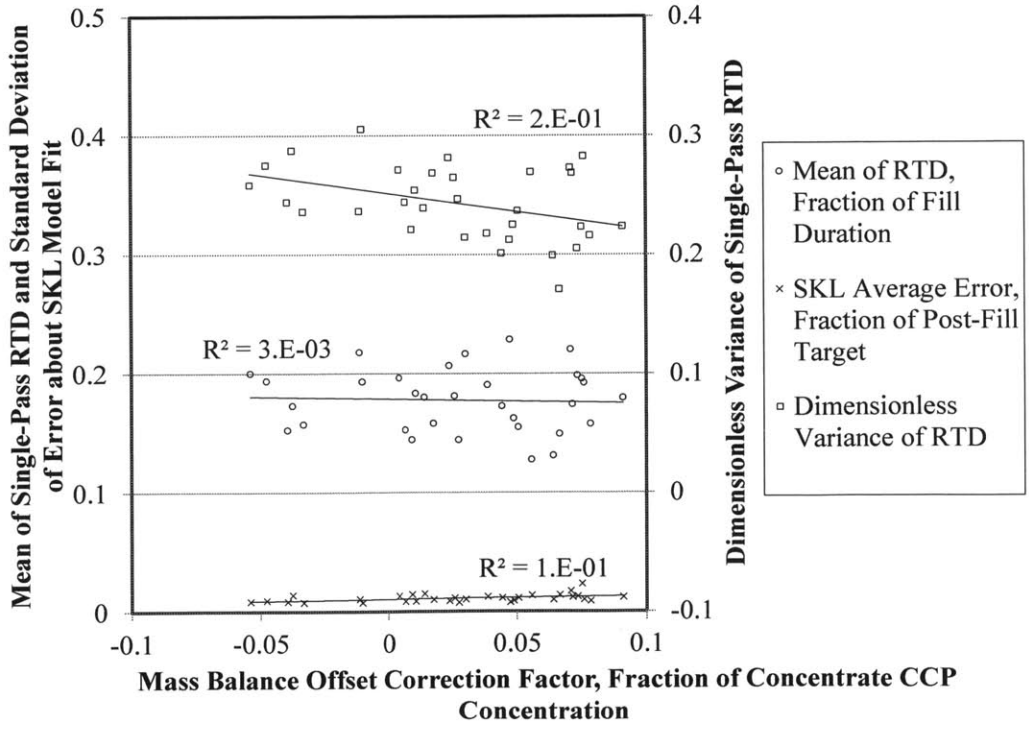

Figure 14 **Transient Concentration Profile Model Parameters vs. Mass Balance Offset Correction**

Finally, the existence of correlations between the transient concentration profile model parameters, which may need to be accounted for in the Monte Carlo modeling, is investigated. The relationship between the mean and dimensionless variance, T and σ_θ^2, respectively, is illustrated in Figure 15. From this plot, there does not appear to be a significant relationship between these parameters, indicating that, for example, the single-pass RTD does not broaden relative to its mean as the mean increases. The relationship between the single-pass RTD parameters and the standard deviation of the error of the model fit is shown in Figure 16. There does not appear to be a significant relationship between the RTD parameters and the variability of the error of the fit.

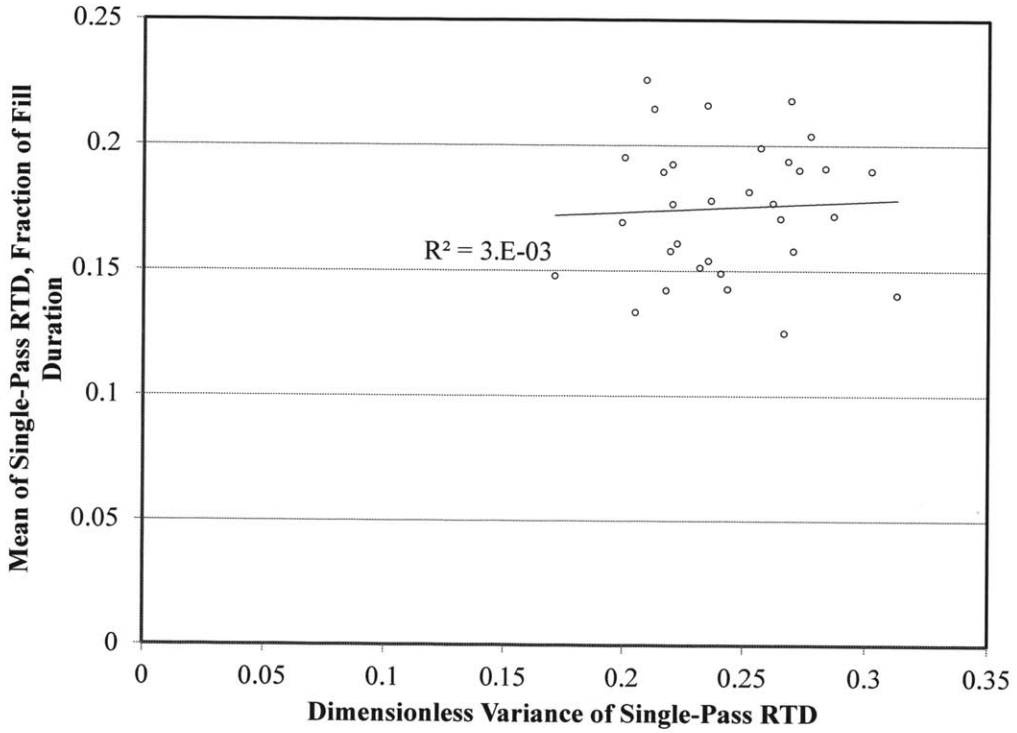

Figure 15 Mean of Single-Pass RTD vs. Dimensionless Variance of Single-Pass RTD

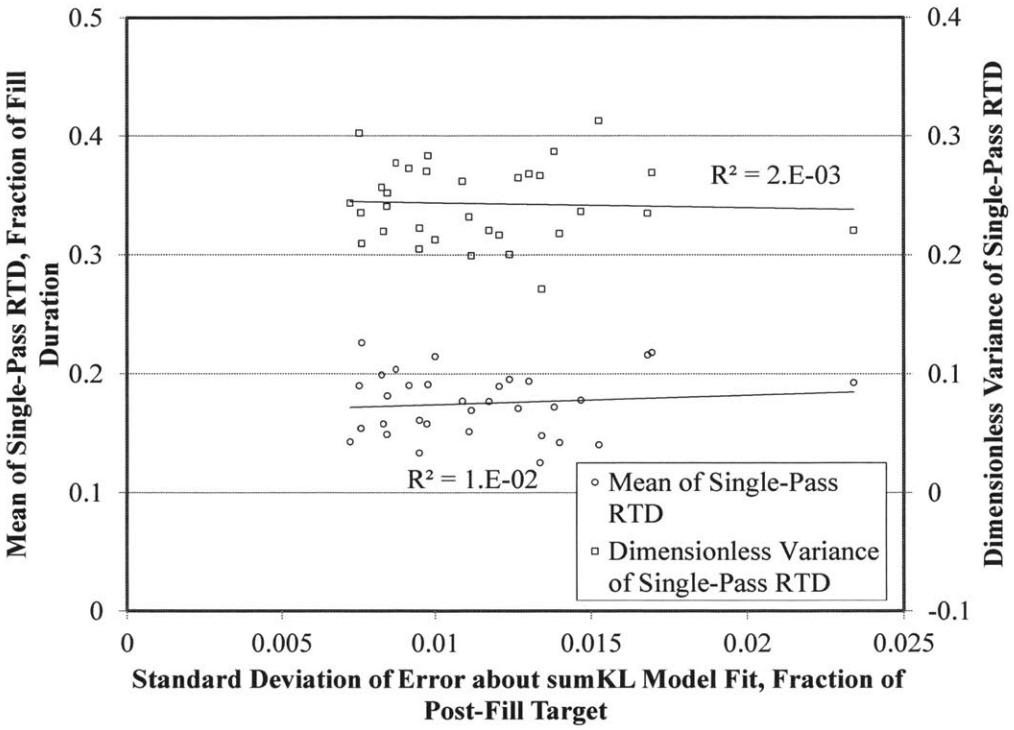

Figure 16 **Single-Pass RTD Parameters vs. Standard Deviation of Error about SKL Model Fit**

6. *PROBABILISTIC ESTIMATION OF VARIABILITY OF FILL OPERATION CONTROL USING MEASURED CCP CONCENTRATION*

For the reasons described in the introduction to Chapter 3, a Monte Carlo simulation approach is used to predict the performance of concentration-based control. The transient concentration profile model, which serves as the foundation of the Monte Carlo simulation, is described in Chapter 5. The development of the Monte Carlo framework used to predict the performance of concentration-based control is described in this chapter. It is noted that the results described in this chapter were generated using the Monte Carlo input parameter values given in Table 1. Following detailed verification of the calculations used to generate this input set, it was necessary to make minor changes. It was determined that these changes did not affect the conclusions drawn in this chapter. Numerical values cited in the text were updated as needed to reflect adjustments to the input set. For readability, Table 1 is given in Section 6.2.1, the section in which its development is described.

6.1. PROBABILISTIC MODELING METHODOLOGY

The Monte Carlo framework is used to simulate a number of Production Reactor fills, where each simulated fill is referred to as a Monte Carlo trial, in which the concentration-based control strategy is applied. The Monte Carlo simulation is built in Microsoft Visual Basic for Applications (VBA), because this software is readily available for use by Amgen. The simulation code is proprietary to Amgen and is withheld from this document.

The structure of the framework is illustrated in Figure 17 and is described as follows. At the start of each Monte Carlo trial, the Production Reactor is modeled to contain a fixed initial volume of liquid and the values of selected parameters, such as the single-pass RTD parameters, are randomly sampled from appropriately defined probability distributions, which are described in Section 6.2. Each simulated fill consists of a series of equal-duration time steps during which a volume of concentrate is injected into the Production Reactor and mixed. A simulated CCP concentration measurement is made during each time step. When a pre-defined CCP concentration is measured, the flow of concentrate is secured and the simulated mixing process is continued until the end of the trial is reached. At the end of each trial, information related to the trial, such as the steady state CCP concentration in the Production Reactor after its contents have mixed to homogeneity, is recorded. The simulation of the fill operation during each Monte Carlo trial is described in additional detail as follows.

As noted above, the time steps into which each Monte Carlo trial is discretized are of equal duration. The time step duration used in the probabilistic simulation described in this chapter is equal to the approximate frequency at which CCP concentration measurements are available in the historical fill data sets (see Section 5.1). The sensitivity of the results of the simulation to this choice of step duration is discussed in Section 6.3.2. At the start of each time step, a measurement of the CCP concentration in the Production Reactor is simulated. (This simulated measurement is described further in the following paragraph.) If the measured CCP concentration does not exceed the user-defined threshold for concentrate inlet valve closure, a pulse of concentrate is injected into the Production Reactor. The volume of the injected pulse is equal to the product of the step duration and the concentrate flow rate sampled for the trial. Following the simulated injection, this pulse and all pulses injected prior to the current time step are mixed according to the transient concentration profile model given by Equation **(8)**. After the mixing calculation is completed, the next time step is initiated and the above process is repeated

until the threshold CCP concentration is measured. When the threshold CCP concentration is measured during a trial, simulated injection of concentrate into the Production Reactor is secured. Note that under this aspect of the modeling, variability in the closure of the concentrate inlet valve is neglected. A quantification of this variability was not available at the time of this work, but its magnitude is expected to be small relative to the other sources that are explicitly taken into account. Finally, the simulated mixing of the concentrate added prior to valve closure is continued during subsequent time steps until the user-defined trial duration is reached (i.e., the trial is concluded after a set number of time steps has been simulated).

As indicated in the previous paragraph, a simulated measurement of the CCP concentration in the Production Reactor is made at the start of each time step. The variability of the probe measurement during the fill is approximated from the residuals of each of the transient concentration profile model fits described in Section 5.4. Specifically, for each of the historical fill data sets, the variability of the probe measurement is approximated as the standard deviation of the residuals of the model fit during the portion of the fill in which concentrate is entering the Production Reactor. This set of standard deviations is used to define the distribution of this parameter in Section 6.2. As noted in Section 5.4, the residuals of the fit are normally distributed. Thus, the variability of the CCP concentration measurement during the fill is modeled as normally distributed scatter about the modeled CCP concentration at the start of the interval.

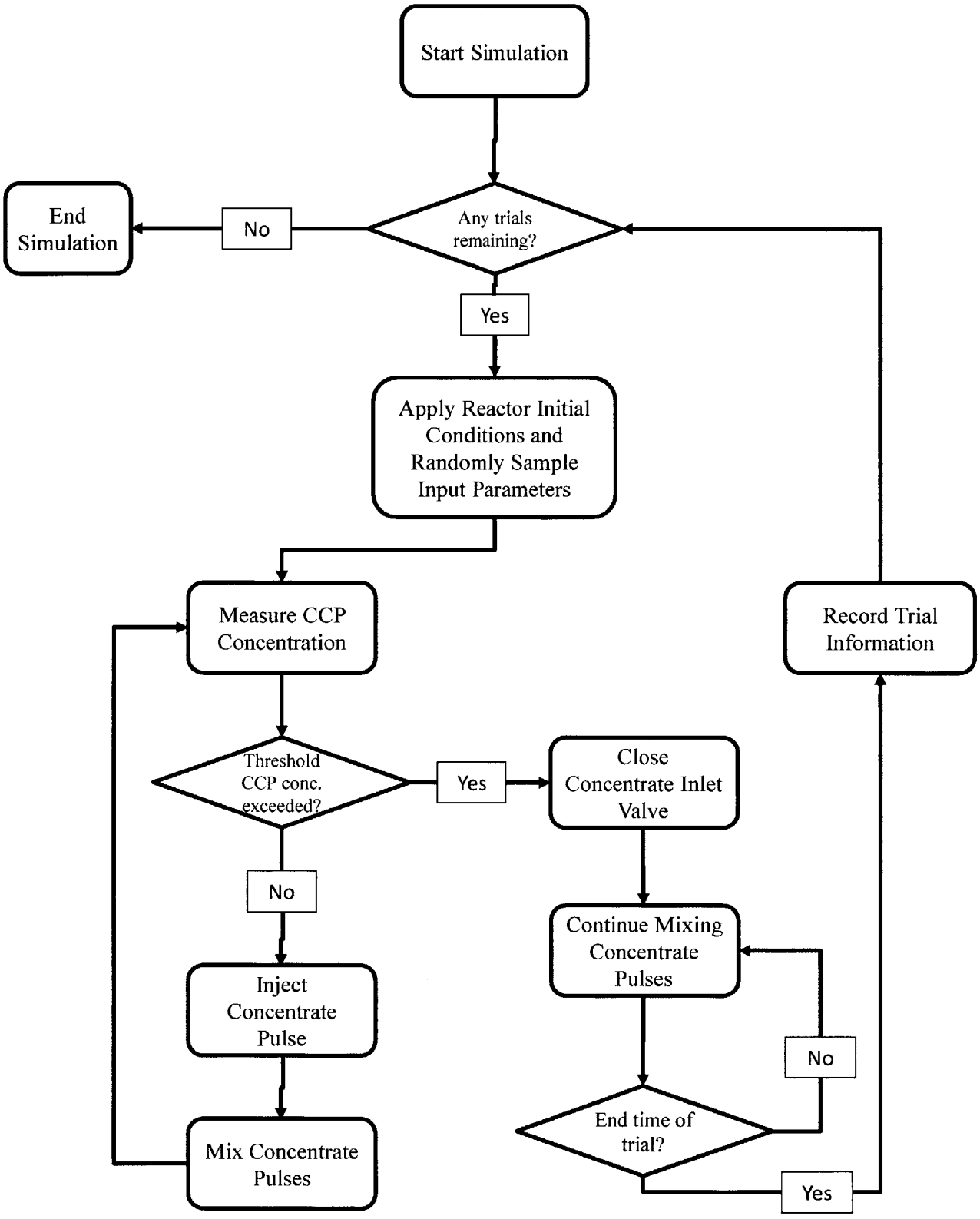

Figure 17 Structure of Monte Carlo Simulation

6.2. *Probabilistic Model Input Parameters and Model Baselining*

As part of the development of a Monte Carlo simulation, it is necessary to develop a suitable set of input parameters and parameter distributions. Before using the model for predictive purposes, it is necessary to verify that the model provides a representative simulation of the behavior of the real system. The development of the set of input parameters is described in Section 6.2.1 and the baselining of the model against the behavior of the real system is described in Section 6.2.2.

6.2.1. Probabilistic Model Input Parameters

At the start of each Monte Carlo trial, the following model parameters are randomly sampled: (1) the initial volume of liquid in the Production Reactor, (2) the concentrate flow rate, (3) the CCP concentration of the source vessel, (4) the single-pass RTD parameters, T and σ_θ^2, and (5) the probe measurement variability during the fill. The probability distributions from which these parameters are sampled are derived from the results of the transient concentration profile modeling described in Chapter 5 (see Appendix B). The development of the distributions for each of these parameters is described in the following paragraphs. Table 1 contains a summary of information related to the Monte Carlo input parameters, which include selected constants, such as the threshold measured CCP concentration at which the flow of concentrate is secured, and representative distributions used for each of the randomly sampled parameters.

The threshold measured CCP concentration at which the flow of concentrate is secured is taken to be the current target post-fill CCP concentration.

The fits of the chosen distributions to the data derived from the modeling described in Chapter 5 are presented in Appendix B . The appropriateness of the selected distributions is judged as follows: (1) the shape of the distribution provides the desired representation of the system of interest, and (2) the distribution provides a visually reasonable fit to the data. The distributions chosen for each parameter are all considered to provide visually reasonable fits. Therefore, this criterion is not discussed further. The following observations are made with respect to the distributions chosen for each parameter.

The normal distribution is used to describe the run-to-run variability of the initial liquid volume in the Production Reactor, concentrate flow rate, and source vessel CCP concentration. Given that the data sets for these parameters are reasonably symmetrically distributed about a

central value, a symmetric distribution, such as a normal distribution is considered appropriate. It is noted that due to the unbounded nature of the normal distribution, it is possible for a randomly sampled value drawn from a normal distribution to be less than zero, which is a nonphysical outcome for these parameters. The normal distributions developed for these parameters are thus truncated at plus-or-minus six sigma bounds. That is, if a value obtained through random sampling is more than six standard deviations above or below the mean, the distribution is resampled until a value falling within the six standard deviation limit is obtained.

The lognormal distribution is used to describe the run-to-run variability of the dimensionless variance of the single-pass RTD and the CCP probe measurement variability. The distributions of these data sets are asymmetric with probability density skewed towards zero and a tail extending away from zero. A lognormal distribution may be used to model such behavior. The lognormal probability distribution is zero for values less than zero and is unbounded in the direction of positive infinity. This behavior is physically appropriate for the CCP probe measurement variability and as such, the distribution for this parameter is not truncated. The lower and upper physical limits of the dimensionless variance of the single-pass RTD are set by the ideal plug flow and stirred tank reactors, respectively, which represent the minimum and maximum possible variances of any RTD [60]. Specifically, the variance of the RTD for an ideal plug flow vessel is zero, which corresponds to a dimensionless variance of zero [61]. The standard deviation of the RTD for an ideal stirred tank is equal to the mean of the RTD [62], which corresponds to a dimensionless variance of unity. Due to the use of a lognormal distribution, which is already bounded by zero, it is not necessary to truncate the lower end of the distribution. The upper end of the distribution is, however, truncated at unity. Given the parameter values determined for the lognormal distribution of the dimensionless variance, the probability that a randomly sampled value would exceed unity is considered to be negligible. Thus, the impact of this truncation is considered to be negligible.

The Weibull distribution is used to describe the run-to-run variability of the mean of the single-pass RTD. The asymmetric nature of the Weibull distribution is appropriate given the shape of the distribution of the data. Similar to the lognormal distribution, the Weibull probability distribution is also zero for values less than zero and is unbounded in the direction of

[60] Schmidt, *The Engineering of Chemical Reactions / Lanny D. Schmidt.*
[61] Ibid.
[62] Ibid.

positive infinity. This lower bound behavior is appropriate for the mean of the RTD as negative time is nonphysical in this context.

Table 1 Summary of Monte Carlo Simulation Inputs

Parameter					Truncation Limits		
Name	Symbol	Distribution Type	Value 1*	Value 2*	Lower	Upper	Comments
Time Step Duration	N/A	Constant	~1%	N/A	N/A	N/A	Fraction of average of mean of single-pass RTD
Initial Volume	N/A	Normal	1.0	0.0037	0.98	1.02	Fraction of current state average value
Concentrate Flow Rate	N/A	Normal	1.0	0.023	0.86	1.1	Fraction of current state average value
Concentrate CCP Concentration	X_S	Normal	1.0	0.055	0.67	1.3	Fraction of current state average value
Threshold CCP for Valve Closure	N/A	Constant	0.97	N/A	N/A	N/A	Fraction of target post-fill CCP concentration
Mean of Single-Pass RTD	T	Weibull	0.19	7.6	N/A	N/A	Value 1 expressed as fraction of current state average fill duration
Dimensionless Variance of Single-Pass RTD	σ_θ^2	Lognormal	-1.4	0.13	N/A	1	N/A
Standard Deviation of SKL Model Fit Error	N/A	Lognormal	-4.5	0.26	N/A	N/A	Fraction of target post-fill CCP concentration

*Legend:
For normal distribution, Value 1 and Value 2 are mean and standard deviation, respectively.
For lognormal distribution, Value 1 and Value 2 are log-mean and log-standard deviation, respectively.
For Weibull distribution, Value 1 and Value 2 are Weibull scale and shape, respectively.

6.2.2. Probabilistic Model Baselining

Following the development of a set of input parameters, it is necessary to evaluate whether the Monte Carlo simulation provides a representative description of the behavior of the real system. To facilitate baselining of the model to the behavior of the real system, the threshold CCP concentration at which the concentrate inlet valve is closed is selected such that the simulation predicts an average steady state CCP concentration that is equal to the average CCP concentration realized during the historical fill data sets from which the simulation inputs are derived. The CCP concentration increase after concentrate inlet valve closure predicted using the simulation is then compared to the same metric recorded for the real system. This metric

provides an appropriate means of comparing the simulation results to the real system, because: (1) it enables a comparison of the simulated mixing process with the real mixing process, which is a fundamental aspect of the transient concentration profile modeling described in Chapter 5 of this document, and (2) it is independent of the process control strategy governing the closure of the valve. It is noted that this metric is not perfectly comparable between the real and the simulated systems. That is, the increase in CCP concentration for the real system is determined as the difference between the measured steady state post-fill CCP concentration and the concentration measured at the time that the flow of concentrate is secured for each of the historical data sets. The concentration increase for the simulated system is determined as the difference between the steady state post-fill concentration and the simulated "true" concentration at the instant the flow of concentrate is secured.

The distributions of the increase in CCP concentration after valve closure for the real and simulated systems are presented in Figure 18. The simulated transient concentration profile is considered to provide an acceptable representation of the real process, because: (1) the centers of the simulated and real distributions are similar, and (2) the spreads of the simulated and real distributions are of similar magnitude. This result indicates that the simulation provides an appropriate balance between the rates of mixing and injection of CCP species.

It is noted that the standard deviation of the simulated distribution, 0.7% of the post-fill target CCP concentration, is less than that of the real distribution, 1.3% of the post-fill target CCP concentration. This discrepancy may be a result of the nature of the data sets used to generate the distributions presented in Figure 18, because: (1) for some of the historical fill data sets, a CCP concentration measurement is not available at the instant the flow of concentrate is secured, which introduces variability that is not reflected in the simulation, and (2) CCP concentration measurement variability, which is reflected in the real distribution, is not reflected in the simulation. Nonetheless, it is conservative to incorporate this additional variability into the predicted standard deviation when developing an operating approach. This potentially nonconservative result is addressed in the recommendations made in Section 6.4.

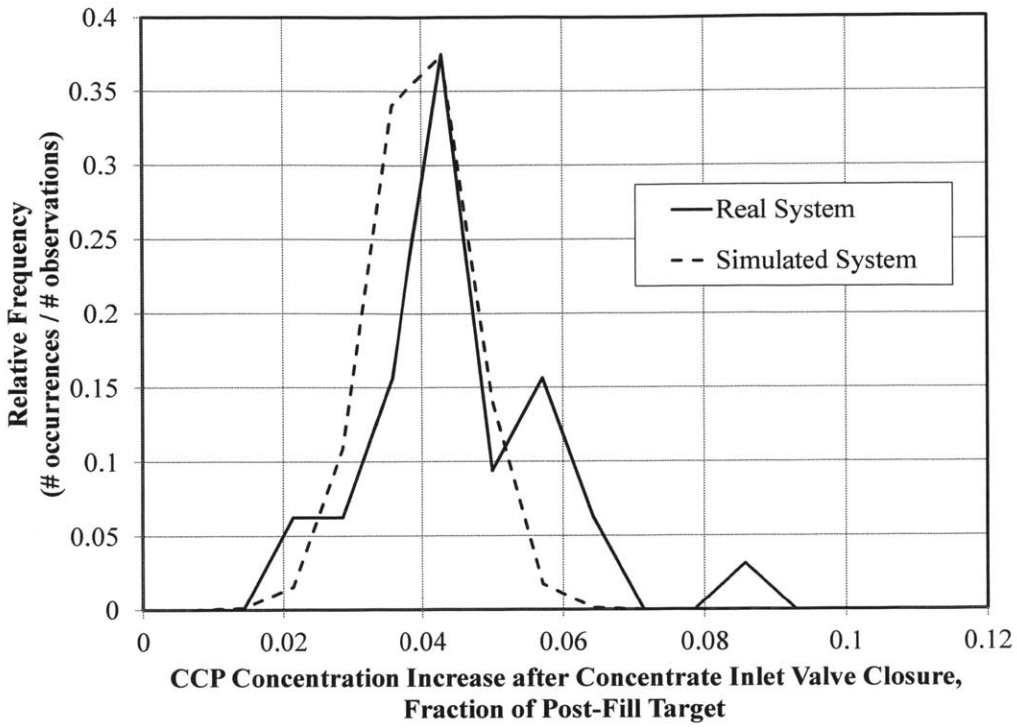

Figure 18 Increase in CCP Concentration after Concentrate Inlet Valve Closure

6.3. Results of Process Performance Simulation

The Monte Carlo simulation developed in the previous sections of this chapter is used to predict the performance of the concentration-based process control strategy. Simulation results obtained using the base case parameters given in Table 1 are presented and discussed in Section 6.3.1. Sensitivities of the Monte Carlo formulation are evaluated in Section 6.3.2.

6.3.1. Base Case Results

The distribution of the realized post-fill CCP concentration from the historical fills, in which the volume-transfer-based process control strategy is used, and the distribution of the simulation results, in which concentration-based control is used, are presented in Figure 19. The following observations are made with regard to the figure. First, the means of the real and the simulated distributions are equal. Second, the simulated distribution is significantly narrower than the real distribution, suggesting that the process control change would provide the desired benefit of reduced variability. The standard deviation of the simulated distribution is approximately 1.1% of the target post-fill CCP concentration, amounting to an approximately

70% reduction in variability relative to the current state (see Section 4.2). This improved control would enable cell culture optimization, such as an increase in the target CCP concentration to increase titer. In the following discussion, the mean and standard deviation are used to characterize the central tendency and spread of these distributions.

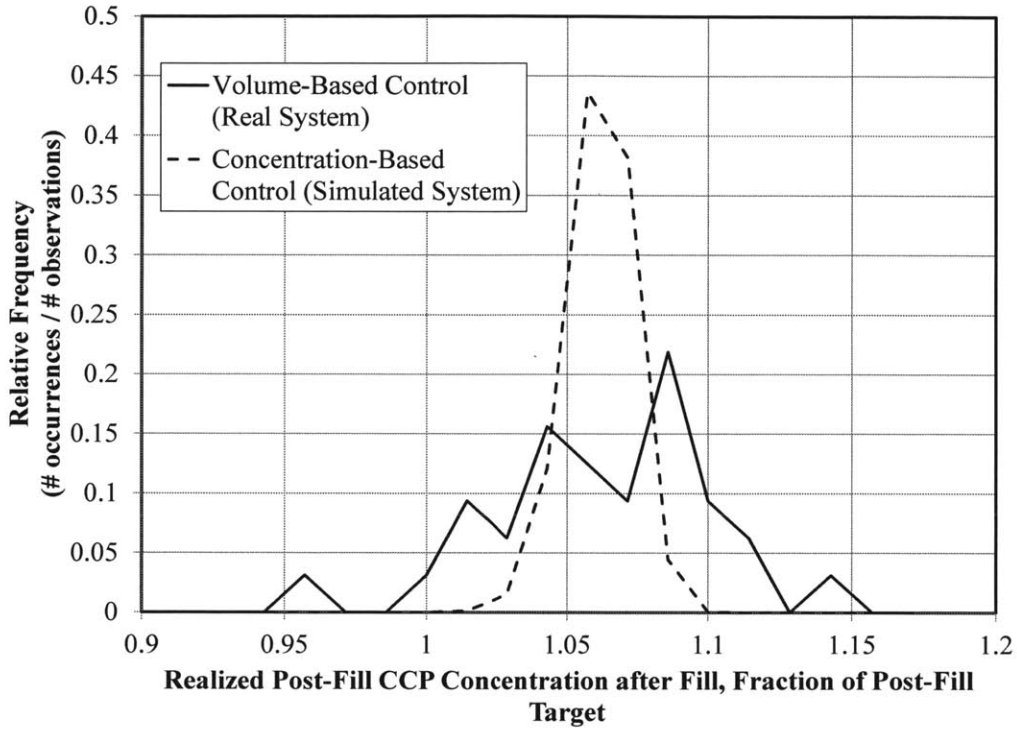

Figure 19 Distribution of Realized Steady State CCP Concentration using Volume-Based and Concentration-Based Process Control

To facilitate the use of this process control strategy for optimization of the fill operation, it is necessary to understand the relationship between the threshold concentrate inlet valve closure CCP concentration and the: (1) mean post-fill CCP concentration, and (2) standard deviation of the post-fill CCP concentration. These quantities are plotted as functions of threshold CCP concentration in Figure 20 and Figure 21. From Figure 20 it is observed that the realized steady state CCP concentration varies proportionally with valve closure threshold over the range of interest, indicating that a linear relationship is suitable for use in defining an operating strategy. From Figure 21 it is observed that the standard deviation in the realized post-fill CCP concentration remains steady over the range of interest, indicating that constant variability may be assumed in the analysis described in Chapter 7. The variation about the line of

best fit presented in Figure 21 is attributed to run-to-run variability in the Monte Carlo results, which is discussed further in Section 6.3.2.

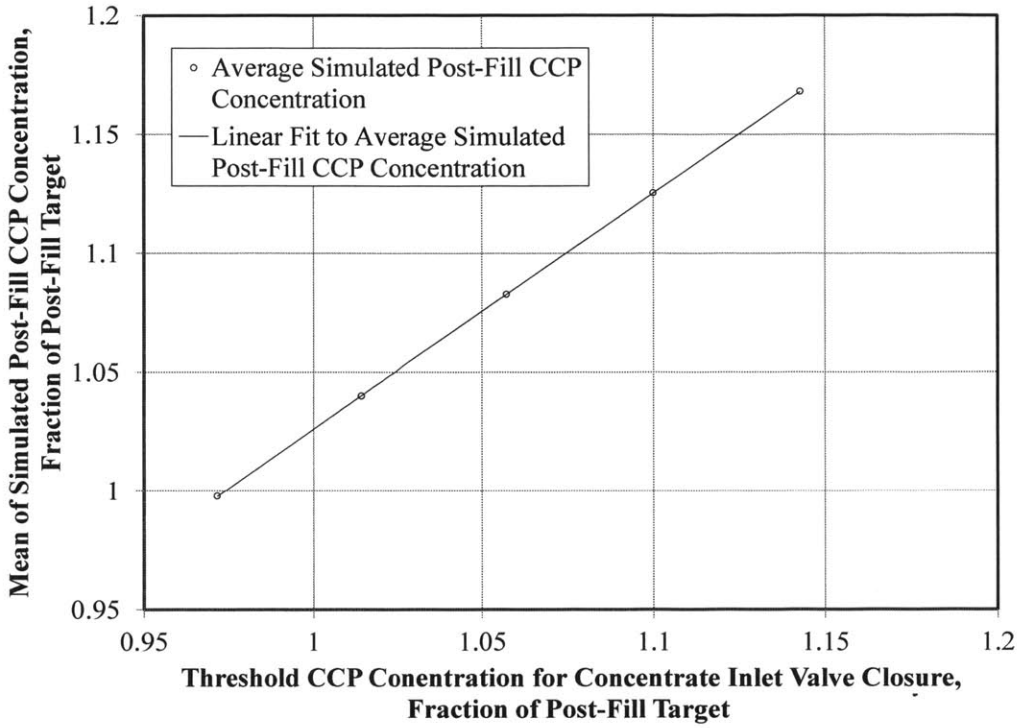

Figure 20 Relationship between Mean of Simulated Performance and Threshold CCP Concentration for Concentrate Inlet Valve Closure

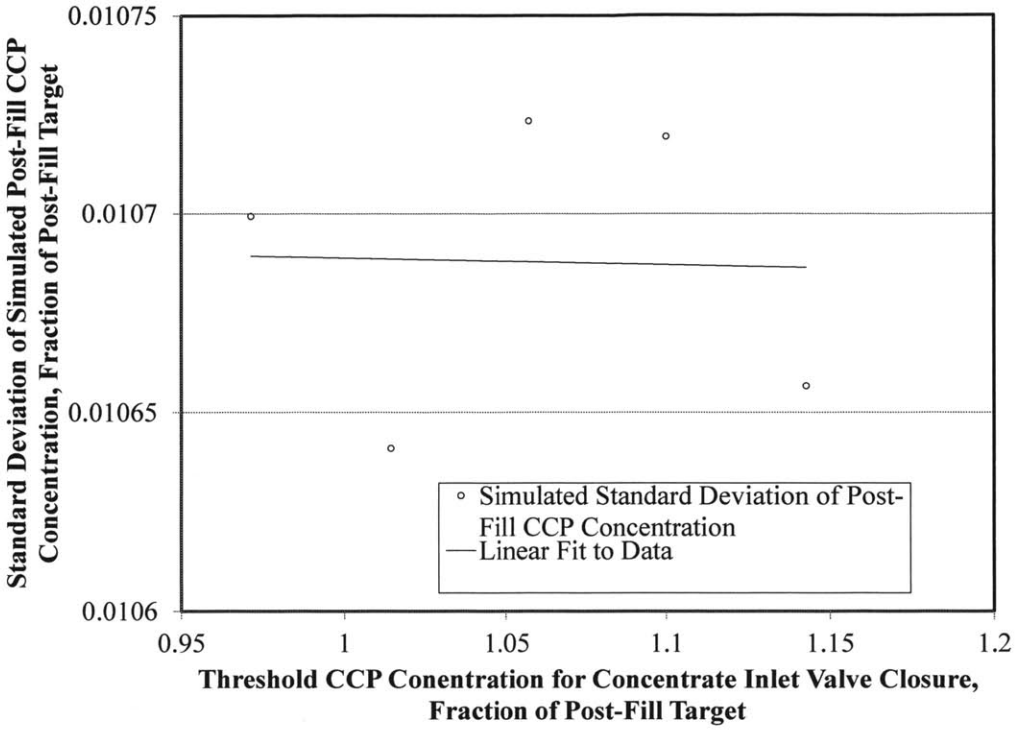

Figure 21 Relationship between Standard Deviation of Simulated Performance and Threshold CCP Concentration for Concentrate Inlet Valve Closure

6.3.2. SENSITIVITY ANALYSIS

The results presented in the previous section were generated using the base case parameter set described in Section 6.2.1. The sensitivity of the results of the Monte Carlo simulation to selected inputs is evaluated in this section.

To understand the variability between Monte Carlo runs when the same the set of inputs is used, the simulation is run ten times. The base case parameter set is used for these runs, with the exception that the threshold CCP concentration for concentrate inlet valve closure is chosen such that the target CCP concentration will be reached rather than the average CCP concentration of the historical fills. The results of these simulations are used to define a basis to which the sensitivity cases may be compared. A single input is varied relative to the base case in each sensitivity case. A description of each sensitivity case is provided in Table 2.

The rationale for the selection of each sensitivity case is described as follows:

- Reduced Concentrate Flow Rate – Further reducing the variability of the fill operation is desirable. It is expected that such a reduction may be achieved by slowing the rate of concentrate injection, as such a change would reduce the rate of

addition of CCP species relative to the rate of mixing, improving the responsiveness of the control strategy.

- Reduced Source Vessel CCP Concentration – It may be desirable to reduce the CCP concentration of the suspension added to the Production Reactor from the source vessel (i.e., the concentrate), for example, to reduce raw material scrap associated with the preparation of the concentrate.
- Increased Source Vessel CCP Concentration – It may be desirable to increase the CCP concentration of the concentrate, for example, to reduce the volume of concentrate required to reach the Production Reactor target post-fill CCP concentration.
- Shorter Simulation Time Step Length – The selection of the time step duration used in the simulation is described in Section 6.1. Shortening the time step has the effect of increasing the frequency of the simulated CCP concentration measurement. This case is of interest under conditions in which the automation system evaluates the CCP concentration measurement more frequently.
- Longer Simulation Time Step Length – Lengthening the time step has the effect of reducing the frequency of the simulated CCP concentration measurement. This case is of interest under conditions in which the automation system evaluates the CCP concentration measurement less frequently.

The results of the sensitivity cases are presented alongside the results of the base case in Figure 22 and Figure 23. The dashed lines presented in Figure 22 and Figure 23 represent two standard deviations below and above the mean performance obtained from the ten simulation runs performed using the base case inputs. Sensitivity cases whose results fall outside of this band are considered to be statistically significantly different from the base case.

The following observations are made with respect to the sensitivity case results. First, the average simulated CCP concentration resulting from all of the sensitivity cases is statistically significantly different from the performance of the base case, but the magnitude of these differences from the base case is small. Second, the standard deviation of the simulated CCP concentration for the low flow and low source concentration cases is less than that simulated for the base case and the variability is higher for the high source concentration case. These results are in agreement with expectation as the effects of mixing variability would be dampened when the CCP species injection rate is reduced and would be more pronounced when the mass injection rate is increased. Finally, while the duration of the simulation time steps appears to have an effect on the simulation results, the magnitude of this effect is small over the evaluated range of interest. Since the standard deviation of the post-fill CCP concentration is the quantity

of primary interest, the time step duration used in the base case input set is considered to be acceptable for the intended use of the simulation.

Table 2 Description of Monte Carlo Sensitivity Cases

Sensitivity Case #	Name	Description
1	Reduced Concentrate Flow Rate	Distribution mean and truncation limits reduced by 25%
2	Reduced Source Vessel CCP Concentration	Distribution mean and truncation limits reduced by 10%
3	Increased Source Vessel CCP Concentration	Distribution mean and truncation limits increased by 10%
4	Shorter Simulation Time Step	Time step reduced by 50%
5	Longer Simulation Time Step	Time step increased by 100%

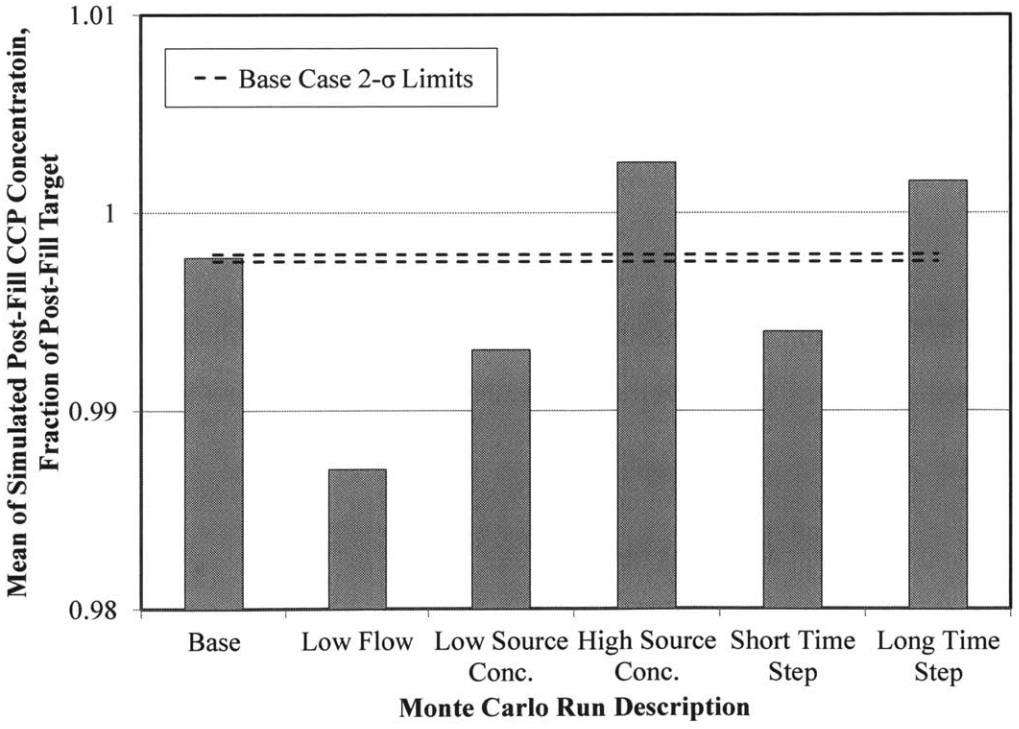

Figure 22 Sensitivity Case Results: Mean of Simulated Performance

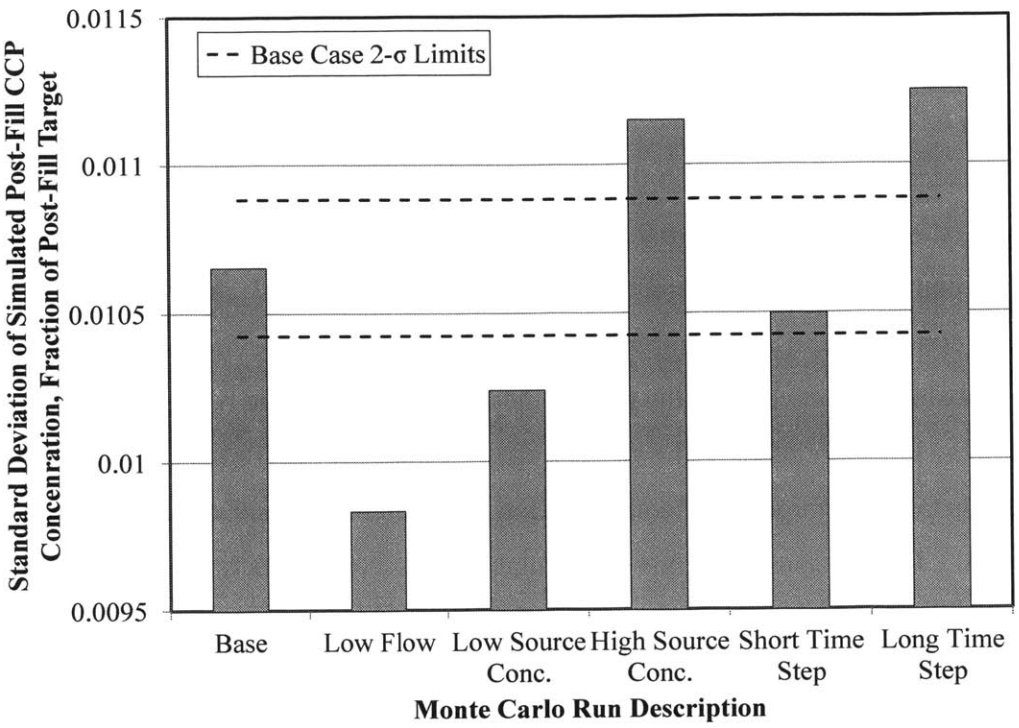

Figure 23 Sensitivity Case Results: Standard Deviation of Simulated Performance

6.4. Recommended Process Control Strategy

Based on the discussion presented in this chapter, it is predicted that concentration-based control will provide a significant reduction in the variability of the performance of the fill operation. A methodology for determining an appropriate increase in the target post-fill CCP concentration is provided in Chapter 7. Provided that the economics for implementing this technology are favorable, it is recommended that concentration-based control be implemented.

The approach taken to generate the information presented in this chapter is considered to be rigorous and appropriate given the nature of the available data and the features of the system of interest. Nonetheless, the simulation results have not been validated using data generated from Production Reactor fills in which concentration-based control is applied. Additionally, while the simulation results are expected to be representative of the performance of the real system, the spread of the simulated distribution of the increase in CCP concentration after concentrate inlet valve closure is less than that of the real system. It is therefore recommended that, should concentration-based control be implemented, a conservative target post-fill CCP concentration be selected and maintained until the true magnitude of the performance improvement may be

demonstrated after a sufficient number of production runs. Following the characterization of the realized performance, it is recommended that an increase in the target post-fill CCP concentration be considered.

If the additional variability in the increase in CCP concentration not predicted by the simulation, as described in Section 6.2.2, is added (by summing variances) to the predicted variability of concentration-based control, described in Section 6.3.1, the predicted standard deviation for concentration-based control is less than the standard deviation of the current state. Thus, the probability of exceeding the pre-defined upper limit for the realized CCP concentration would not be increased relative to the current state if concentration-based control is implemented and the current post-fill target CCP concentration is maintained. Thus, maintaining the current target post-fill CCP concentration may be a suitably conservative operating approach.

7. *Quantification of Benefit of Reducing Variability*

As described throughout this document, variability is of particular interest in the Production Reactor fill operation and technologies that enable this variability to be reduced are valuable. The variability of the performance of the current state fill operation is characterized in Chapter 4. A prediction of the variability of the proposed concentration-based process control strategy is developed in Chapters 5 and 6. Using this information, the impact of concentration-based process control on the performance of the fill operation may be estimated.

A methodology for assessing the economic impact of reducing the variability of the fill operation is described in Section 7.1. An application of this framework to a hypothetical scenario in which the productivity of the protein production step is directly proportional to the post-fill CCP concentration in the Production Reactor is provided for demonstrative purposes in Section 7.2. A brief discussion of how this methodology may be applied in the business evaluation of a technology that provides a reduction in variability of the fill operation is given in Section 7.3.

7.1. *Optimization Methodology for Selecting Target CCP Concentration*

As described in Section 3.1, the Newsvendor Problem is a well-known concept in the subject of operations management (see [63] and [64], for example). The Newsvendor framework

[63] McClain and Thomas, *Operations Management : Production of Goods and Services / John O. McClain, L. Joseph Thomas.*

provides a methodology with which a newspaper vendor may determine the number of newspapers that he or she should buy at the beginning of the newsday to maximize expected profit. This framework is based on the probability distribution of demand for newspapers, the lost revenue resulting from a stockout (i.e., running out of papers to sell), and the cost associated with failing to sell all of the papers, the purchase price less the revenue from recycling unsold papers, for example. The Newsvendor framework is reproduced from [65]:

$$\begin{aligned}&(net\ revenue + shortage\ cost)[Prob(d \geq Q)] \\ &\qquad = (cost - salvage\ value)[1 - Prob(d \geq Q)]\end{aligned} \tag{12}$$

where d is the realized demand for newspapers, Q is the number of newspapers purchased by the vendor, and revenue, cost, and salvage value are in per-newspaper terms.

For convenience, Equation (12) is expressed in a more compact and general form:

$$R_1 \times p = -R_2 \times (1 - p) \tag{13}$$

where R_1 and R_2 are the net revenues of each outcome, respectively, and p is the probability of occurrence of the R_1 net revenue outcome. In this form, it is apparent that Equation (13) represents the balance between two outcomes having given probabilities of occurrence and given net benefits (costs). The Newsvendor approach is applicable to the many types of problems that fit these criteria, such as the selection of a Production Reactor target post-fill CCP concentration.

The adaptation of the Newsvendor framework to the Production Reactor fill operation is developed in the following discussion. For clarity, it is noted that the problem of interest is not a Newsvendor problem. The Newsvendor approach is applied, because the mathematical behavior of the problem of interest is similar to that of the Newsvendor problem.

Maximizing the expected profit of the manufacturing process by optimizing the target post-fill CCP concentration is a reasonable objective and is chosen as the starting point for this discussion. A generic equation for expected profit is given as follows:

$$\begin{aligned}E[profit] &= E[revenue] - E[cost] \\ &= E[revenue] - (number\ of\ batches) \times E[cost/batch]\end{aligned} \tag{14}$$

where the E operator represents the expected value of the operand.

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[64] Cachon and Terwiesch, *Matching Supply with Demand : An Introduction to Operations Management / Gérard Cachon, Christian Terwiesch.*

[65] McClain and Thomas, *Operations Management : Production of Goods and Services / John O. McClain, L. Joseph Thomas.*

In the pharmaceutical industry, it is often reasonable to assume that a manufacturer will always produce a sufficient quantity of a given therapeutic to meet demand. Indeed, providing a safe and reliable supply of medicine is core to Amgen's mission to serve patients [66]. This assumption is also relevant to the problem of interest. Therefore, expected revenue is considered to be fixed and expected profit is maximized by minimizing expected cost. Adapting the Newsvendor framework to this problem, expected cost per batch is expressed as follows:

$$E[cost/batch] = (FC + VC) \times p + (FC + VC + NC) \times (1 - p) \quad \textbf{(15)}$$

where FC is the per-batch fixed cost, VC is the per-batch variable cost, NC is the cost incurred as a result of exceeding the pre-defined upper CCP concentration limit for the fill operation during a given batch (a nonconformance with procedure), and p is the probability that the realized post-fill CCP concentration will not exceed the pre-defined upper limit during a given production batch. Equation (15) is simplified as follows:

$$E[cost/batch] = FC + VC + NC \times (1 - p) \quad \textbf{(16)}$$

As noted above, an optimal target post-fill CCP concentration may be chosen to maximize expected profit, which in the context of this analysis, is accomplished by minimizing expected cost. From Equations (14) and (16), the expected cost incurred to meet demand is given as follows:

$$E[cost] = (number\ of\ batches) \times [FC + VC + NC \times (1 - p)] \quad \textbf{(17)}$$

The dependence of each term on the RHS of Equation (17) on the target CCP concentration is assessed as follows.

- *Number of batches* – In the example that is presented in this chapter, it is assumed that the product of interest is manufactured to meet demand and that, per Equation (3), the quantity of product resulting from each batch is directly proportional to the realized post-fill CCP concentration. Therefore, increasing the realized post-fill CCP concentration by increasing the target would increase the quantity of product per batch, reducing the number of production batches required to meet demand.
- FC – By definition, the fixed cost of production is independent of production volume. While, in an economic sense, fixed costs become variable in the long run, it is assumed that the timeframe that is relevant to this optimization is sufficiently short that all fixed costs remain fixed. Thus, FC is not a function of target post-fill CCP concentration.

[66] "Amgen - About Amgen - Mission & Values."

- *VC* – The per-batch variable cost, which may include, for example, raw materials and/or the opportunity cost of production time, is assumed to be constant as all batches are nominally the same and the change in overall yield over the range of interest is small.
- *NC* – For simplicity, the cost of a nonconformance is taken to be a constant. This assumption is considered to be reasonable if the number of nonconformances is maintained at a sufficiently low level from the perspective of regulators, the Amgen Quality organization, and other stakeholders. An attempt to quantify this level is not made in this document.
- p – It is reasonable to believe that increasing the target post-fill CCP concentration will lead to an increase in the average realized CCP concentration. This assumption is described below in additional detail. Per the discussion given in Section 6.3.1, it is expected that the variability of the fill operation will remain steady over the range of interest. Thus, the probability that the realized post-fill CCP concentration will exceed the pre-defined limit will increase as the target is increased.

The number of batches needed to meet demand is expressed as follows:

$$number\ of\ batches = d \times \left(\frac{1}{\overline{M_P}}\right) \tag{18}$$

where d is demand (units of mass of product), and $\overline{M_P}$ is the average per-batch yield of the process (units of mass of product per batch).

The relationship between the probability that the realized post-fill CCP concentration will exceed the upper limit, p, and the target CCP concentration may be described using an appropriate method of statistical inference, such as a probability distribution. This relationship is expressed as follows:

$$p = P\left(CCP \leq CCP_{upper} | CCP_{target}\right) \tag{19}$$

where the RHS of the above equation represents the probability that the realized CCP concentration resulting from a given fill will not exceed the pre-defined upper limit for a given target CCP concentration.

Combination of Equations (17), (18), and (19) yields the generic equation for expected cost associated with the fill operation:

$$E[cost] = \left(\frac{d}{\overline{M_P}}\right) \times \left[FC + VC - NC \times \left(1 - P\left(CCP \leq CCP_{upper} | CCP_{target}\right)\right)\right] \tag{20}$$

The optimal target CCP concentration is determined by locating the appropriate optimum on a plot of Equations (19) and (20). The appropriate optimum is determined by the chosen objective, minimizing cost in this case, and relevant constraints. An example application of this methodology is provided in Section 7.2.

7.2. Example Application of Optimization Methodology

As described in Section 7.1, the optimal target post-fill CCP concentration may be determined from a plot of Equations (19) and (20).

To apply Equations (19) and (20), it is necessary to develop relations between target CCP concentration and batch-wise yield and between target CCP concentration and the probability that the realized CCP concentration will not exceed the pre-defined upper limit. For convenience, the relationship between the probability that the realized CCP concentration will not exceed the upper limit, p, and the target CCP concentration is addressed first.

One of the advantages of a Monte Carlo approach is that an arbitrarily large data set, relative to the number of actual production runs executed, may be generated. When a sufficiently large data set is available, it is not strictly necessary to assume a distribution to describe the data (i.e., a nonparametric approach may be applied). However, it is likely that the improvement provided by concentration-based control would need to be demonstrated in the actual system before the target CCP concentration could be increased. Thus, the smaller data set of actual process performance would be used as the basis for justifying an increase in the target rather than the larger, simulation-based data set. Given the substantially smaller size of the data set based on actual system performance, it is expected that parametric inference would be used (i.e., a probability distribution would be assumed).

The results of a base case Monte Carlo run are given in Figure 24. From Figure 24, it is observed that a normal distribution provides a reasonable fit to the simulation results and that it provides a conservative description of the upper end of the distribution (i.e., the probability of occurrence of the greatest realized post-fill CCP concentration data is overpredicted by a normal distribution). (Note that while some of the Monte Carlo inputs are normally distributed (see Table 1), it is not obvious that the simulation results as depicted in Figure 24 would be normally distributed due to the use of non-normal distributions for some other input parameters and the nonlinearity of the transient concentration profile model, described in Chapter 5, upon which the

simulation is built.) Thus, a normal distribution with mean equal to the target post-fill CCP concentration and standard deviation derived from the data presented in Figure 24 is chosen to describe the relationship between p and target CCP concentration. This relationship is given as follows:

$$p = \Phi\left(CCP_{Upper} | CCP_{target}, \sigma_{CCP}\right) \quad \textbf{(21)}$$

where Φ represents the cumulative normal distribution with mean equal to the target CCP concentration and standard deviation equal to the standard deviation of the performance of the fill under concentration-based control and CCP_{Upper} is the pre-defined upper limit CCP concentration.

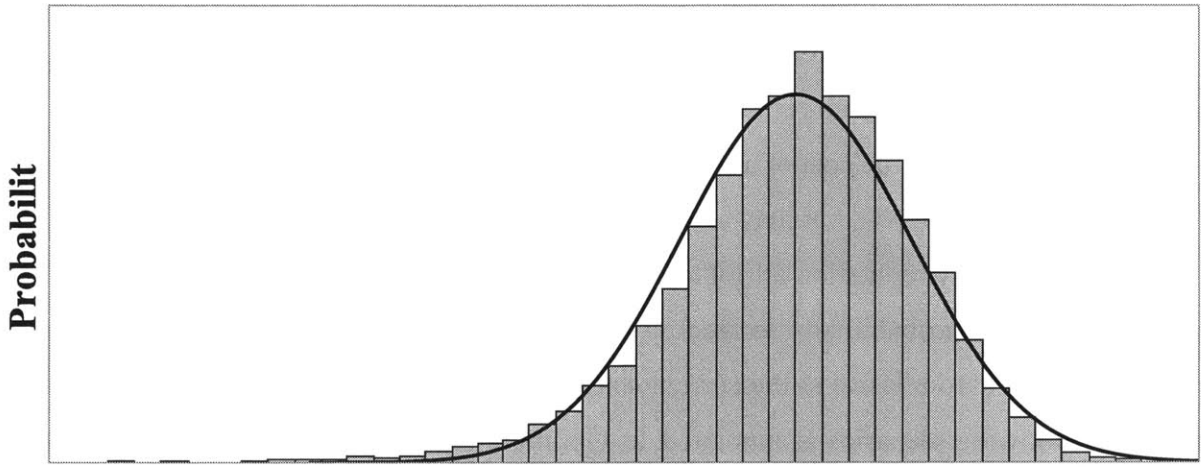

Figure 24 Distribution of Simulated Post-Fill CCP Concentration using Concentration-Based Control with Overlaid Normal Distribution

Given the approximately normal behavior of the system, it is reasonable to state that the average per-batch yield of the process occurs at the target CCP concentration. From Equations (3) and (18), the number of batches required to meet demand is given as follows:

$$number\ of\ batches = d \times \left(\frac{1}{Y \cdot CCP_{target} + constant}\right) \quad \textbf{(22)}$$

From Equations (17), (21), and (22) the expected cost incurred to meet demand is expressed in terms of target CCP concentration as follows:

$$E[cost] = \left(\frac{d}{Y \cdot CCP_{target} + constant}\right) \times \left[FC + VC - NC \times \left(1 + \Phi\left(CCP_{Upper} | CCP_{target}, \sigma_{CCP}\right)\right)\right] \quad (23)$$

An illustrative plot of Equations (21) and (23) is given in Figure 25 to demonstrate the graphical optimization approach. The values of the parameters of Equations (21) and (23) used to generate this plot were selected to provide a readily visually interpretable plot. While the parameter values used to generate the plot are not representative of the actual system, the qualitative relationship between expected cost, probability of exceeding the upper limit CCP concentration, and the target post-fill CCP concentration is demonstrated.

The appropriate optimum target CCP concentration depends on the objective and the constraints of the problem. The objective of minimizing cost is chosen for this example. The first constraint is that the selected target CCP concentration may not exceed the pre-defined upper limit. It is noted that the true cost of a nonconformance includes not only the direct resources consumed to address the NC, but may also include costs that are more difficult to quantify, such as damage to company reputation attributable to frequent NCs. Thus, a second possible constraint is that the probability of exceeding the upper limit CCP concentration may not be intentionally increased relative to the current state. The optimal target CCP concentration for each of the following scenarios is annotated, as Optimum 1 and Optimum 2, respectively, on Figure 25: (1) the scenario in which only the first constraint is enforced, and (2) the scenario in which both constraints are enforced.

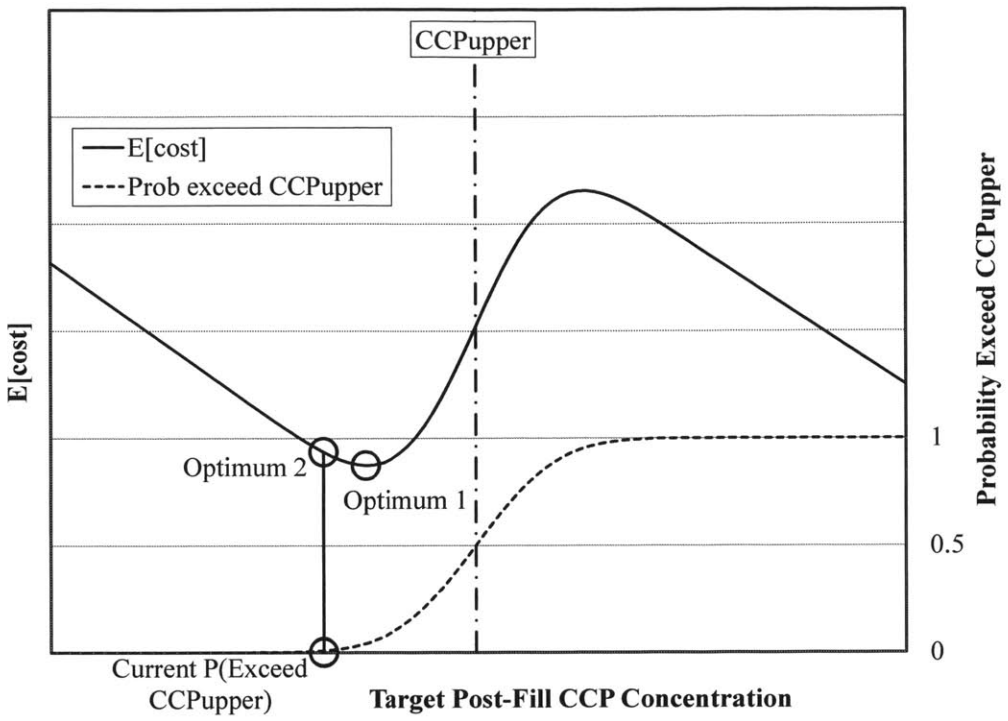

Figure 25 **Demonstration of Graphical Approach to Selecting Optimum Target Post-Fill CCP Concentration (inputs used to generate plot chosen to facilitate ready visual interpretation of optimization method; plot is not representative of Amgen performance)**

7.3. Use of Optimization Methodology to Evaluate Economics of Technologies Affecting Variability

Because the optimization methodology for selecting the target CCP concentration is based on cost, it may be used directly to assess the economics of a fill operation improvement for use in business case development. Specifically, when considering potential fill operation improvements, the following approach is recommended. First, apply the optimization to the current state technology and determine if the current operating approach is indeed optimal. Second, apply the optimization to the potential improvement. The difference between the expected costs of the potential improvement and the current state optimum represents the economic benefit of the improvement. This piece of information is often critical in the development of a business case used to support a process improvement, for example, in a calculation of the net present value of the cash flows provided by the improvement.

8. *Conclusions and Recommendations*

Provided in this document is a discussion of a fill operation taking place during the protein production step of a particular molecule manufactured at the Amgen Rhode Island drug substance manufacturing facility. The body of this document provides a description of the current state of the fill operation, a prediction of the performance of a proposed process improvement, and a general methodology that may be used to quantify the economic value of this process improvement and other improvements involving process variability.

8.1. Conclusions for Manufacturing at Amgen

The following conclusions are drawn based on the work documented in this report.

- The variability of the performance of the Production Reactor fill operation at Amgen Rhode Island may be reduced by implementing concentration-based process control in place of the current state volume-transfer-based strategy. It is predicted that the variability of the performance of this fill operation may be reduced by approximately 70%.
- The batch-wise yield benefit that may be achieved through adoption of this method and subsequent process optimization would provide: (1) reduced variable cost incurred to meet demand, and (2) liberated production time that may be used for other activities, such as production of additional batches of other molecules.
- Modeling the concentration profile in an agitated tank during a sustained filling operation as the sum of a series of pulse injections is feasible under the conditions of interest in this document.
- When appropriate data are available, probabilistic modeling provides a means by which the impact of a process improvement may be investigated. This capability is important in the biopharmaceutical industry where conducting experiments in equipment commissioned for production of commercial therapeutic material is difficult.

8.2. Recommendations for Future Initiatives

The following recommendations for future initiatives are made.

- Implement concentration-based fill operation control in the Production Reactors and/or conduct an appropriately designed experiment to generate data against which the simulation results documented in Chapter 6 may be validated.

- Identify other situations both within Amgen and within the biotechnology industry to which the risk-based methodology for determining process setpoints and evaluating process improvements may be applied.
- Assess how simulation approaches, such as that documented in this work, may be applied within a process development environment to reduce the demand on highly utilized laboratory and pilot plant personnel and resources and/or to reduce the time required to obtain results.

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Appendix A DERIVATION OF EQUATION (8)

As described in Section 5.3, it is convenient to factor the continuous time, t, out of the summation terms of Equation (6) to facilitate fitting of this model to historical data. For convenience, Equation (6) is reproduced from Section 5.2:

$$X(t) = \sum_i \frac{(\Delta V \cdot X_S)_i}{V(t)} \left[1 + 2e^{-K(t-t_i)} cos\left(\frac{2\pi}{T}(t - t_i) + 2\pi\sigma_\theta^2\right)\right] \quad (24)$$

where the variables are defined previously.

The derivation of Equation (8) is begun by expressing the above equation in the following simpler form:

$$X(t) = \frac{1}{V(t)}(A + B) \quad (25)$$

$$A = \sum_i (\Delta V \cdot X_S)_i \quad (26)$$

$$B = \sum_i (\Delta V \cdot X_S)_i \cdot 2e^{-K(t-t_i)} cos\left(\frac{2\pi}{T}(t - t_i) + 2\pi\sigma_\theta^2\right) \quad (27)$$

The B equation is factored as follows:

$$B = \sum_i (\Delta V \cdot X_S)_i \cdot 2e^{-Kt} e^{Kt_i} cos\left(\frac{2\pi}{T}(t - t_i) + 2\pi\sigma_\theta^2\right) \quad (28)$$

$$B = 2e^{-Kt} \sum_i (\Delta V \cdot X_S)_i \cdot e^{Kt_i} \cdot cos\left(\frac{2\pi}{T}(t - t_i) + 2\pi\sigma_\theta^2\right) \quad (29)$$

$$B = 2e^{-Kt} \sum_i (\Delta V \cdot X_S)_i \cdot e^{Kt_i} \cdot cos\left(\frac{2\pi}{T}t + 2\pi\left(\sigma_\theta^2 - \frac{t_i}{T}\right)\right) \quad (30)$$

The cosine term of the above equation is factored using the following trigonometric identity [67]:

$$cos(\alpha + \beta) = cos(\alpha)cos(\beta) - sin(\alpha)sin(\beta) \quad (31)$$

[67] Abramowitz, Stegun, and National Bureau of Standards (DOC), *Handbook of Mathematical Functions with Formulas, Graphs, and Mathematical Tables. National Bureau of Standards Applied Mathematics Series 55. Tenth Printing.*

Using this identity, Equation (30) is factored as follows:

$$B = 2e^{-Kt}\sum_i (\Delta V \cdot X_S)_i \cdot e^{Kt_i} \cdot \left[cos\left(\frac{2\pi}{T}t\right)cos\left(2\pi\left(\sigma_\theta^2 - \frac{t_i}{T}\right)\right) - sin\left(\frac{2\pi}{T}t\right)sin\left(2\pi\left(\sigma_\theta^2 - \frac{t_i}{T}\right)\right)\right] \quad (32)$$

Equation (32) is simplified as follows:

$$B = C - D \quad (33)$$

$$C = 2e^{-Kt}\sum_i (\Delta V \cdot X_S)_i \cdot e^{Kt_i} \cdot cos\left(\frac{2\pi}{T}t\right)cos\left(2\pi\left(\sigma_\theta^2 - \frac{t_i}{T}\right)\right) \quad (34)$$

$$D = 2e^{-Kt}\sum_i (\Delta V \cdot X_S)_i \cdot e^{Kt_i} \cdot sin\left(\frac{2\pi}{T}t\right)sin\left(2\pi\left(\sigma_\theta^2 - \frac{t_i}{T}\right)\right) \quad (35)$$

Equations (34) and (35) are then simplified as follows:

$$C = 2e^{-Kt} \cdot cos\left(\frac{2\pi}{T}t\right)\sum_i (\Delta V \cdot X_S)_i \cdot e^{Kt_i} \cdot cos\left(2\pi\left(\sigma_\theta^2 - \frac{t_i}{T}\right)\right) \quad (36)$$

$$D = 2e^{-Kt} \cdot sin\left(\frac{2\pi}{T}t\right)\sum_i (\Delta V \cdot X_S)_i \cdot e^{Kt_i} \cdot sin\left(2\pi\left(\sigma_\theta^2 - \frac{t_i}{T}\right)\right) \quad (37)$$

Finally, Equation (8) is given by combination of Equations (25), (26), (33), (36), and (37):

$$X(t) = \frac{1}{V(t)}\left[\sum_i (\Delta V \cdot X_S)_i + 2e^{-Kt} \cdot cos\left(\frac{2\pi}{T}t\right)\sum_i (\Delta V \cdot X_S)_i \cdot e^{Kt_i} \cdot cos\left(2\pi\left(\sigma_\theta^2 - \frac{t_i}{T}\right)\right) - 2e^{-Kt} \cdot sin\left(\frac{2\pi}{T}t\right)\sum_i (\Delta V \cdot X_S)_i \cdot e^{Kt_i} \cdot sin\left(2\pi\left(\sigma_\theta^2 - \frac{t_i}{T}\right)\right)\right] \quad (38)$$

Appendix B TRANSIENT CONCENTRATION PROFILE MODEL FITTING RESULTS AND PROBABILITY DISTRIBUTIONS USED IN MONTE CARLO SIMULATION

As described in Section 5.4, the model given in Equation (8) is fit to a number of historical fill data sets according to the approach described in Section 5.3. The fits of the probability distributions described in Section 6.2 to the results obtained from the modeling described in Chapter 5 are presented in this appendix. The rationale for and appropriateness of the selected distributions are described in Section 6.2.1.

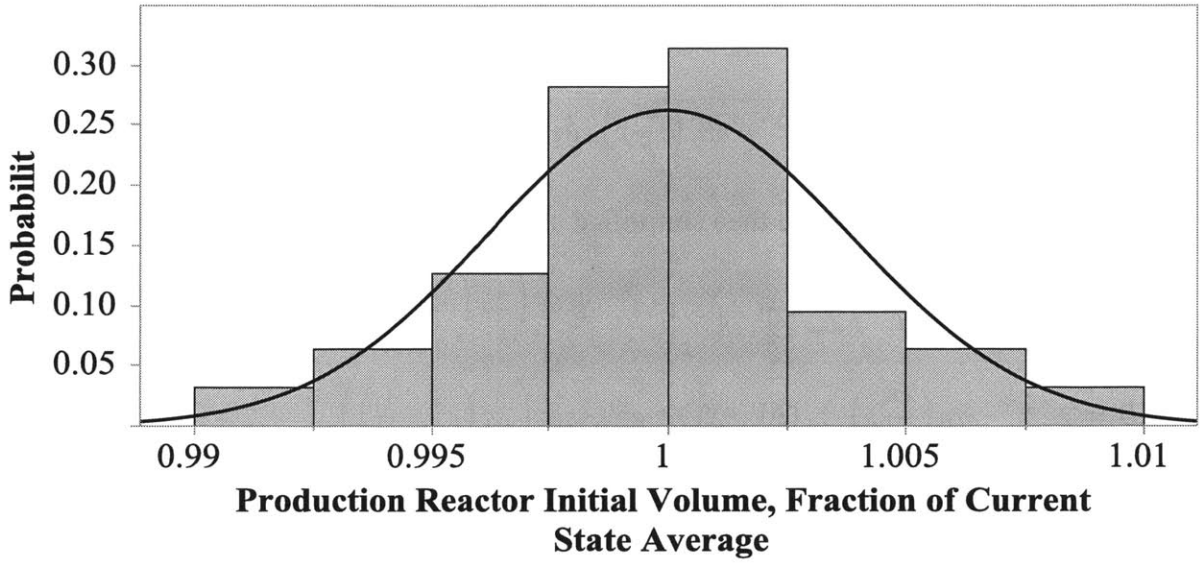

Figure 26 Fitted Normal Distribution of Initial Liquid Volume in Production Reactor

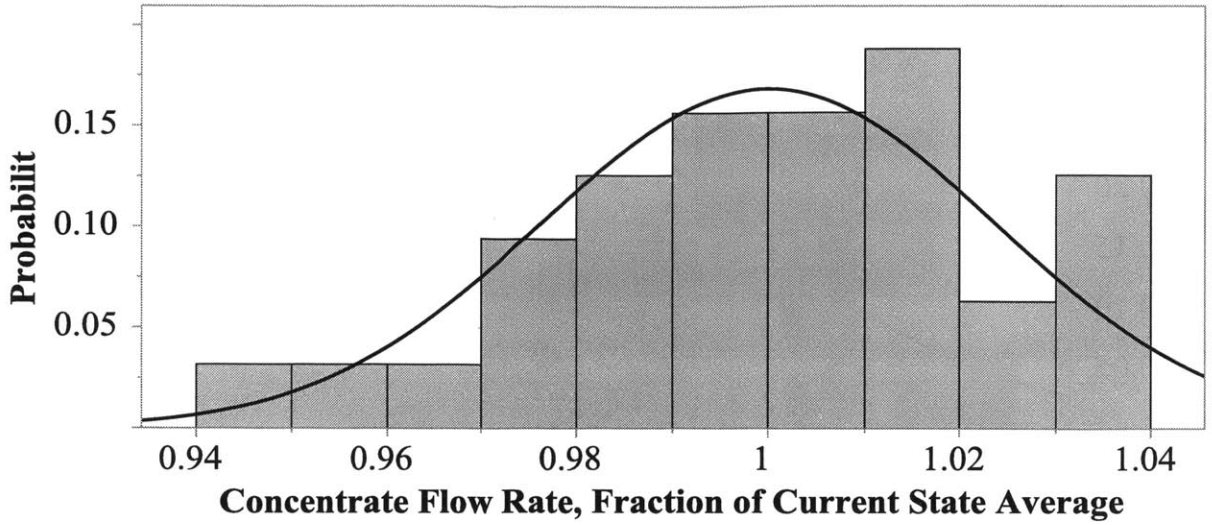

Figure 27 **Fitted Normal Distribution of Concentrate Flow Rate**

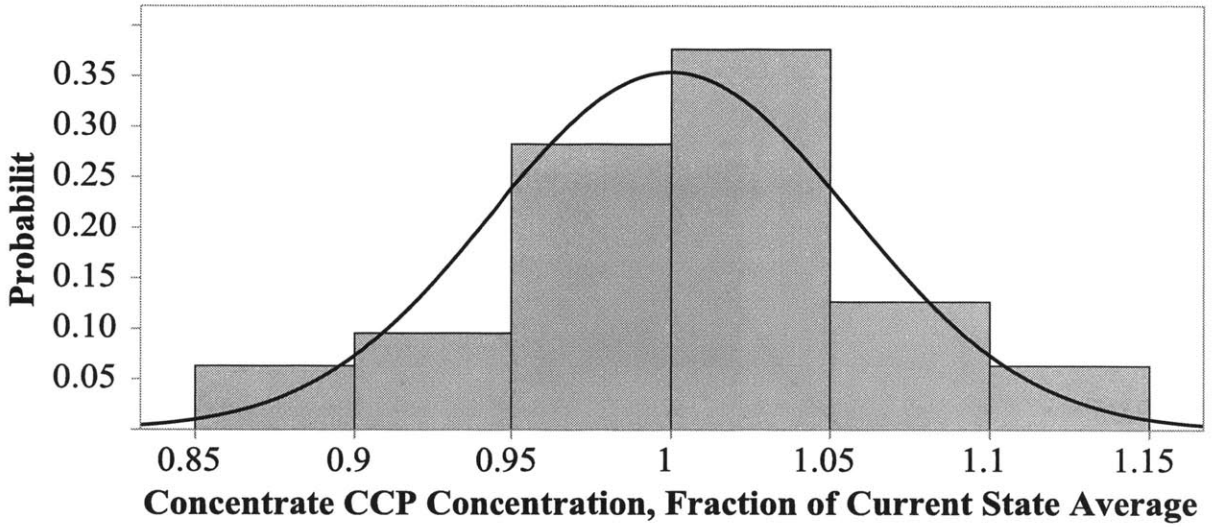

Figure 28 **Fitted Normal Distribution of Source Vessel CCP Concentration**

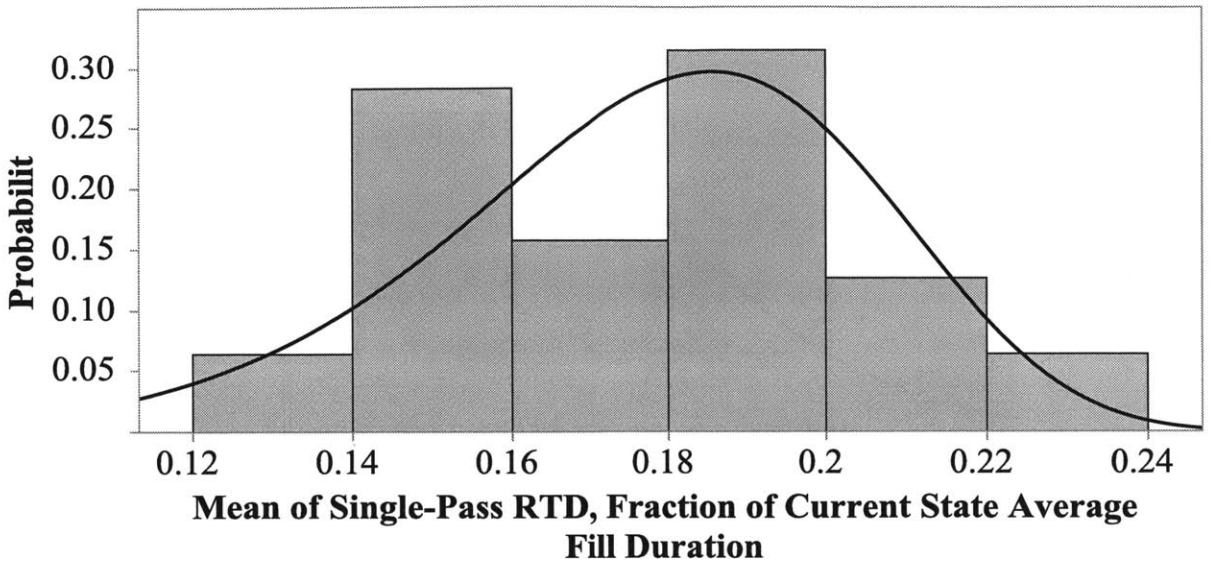

Figure 29 **Fitted Weibull Distribution of Mean of Production Reactor Single-Pass RTD**

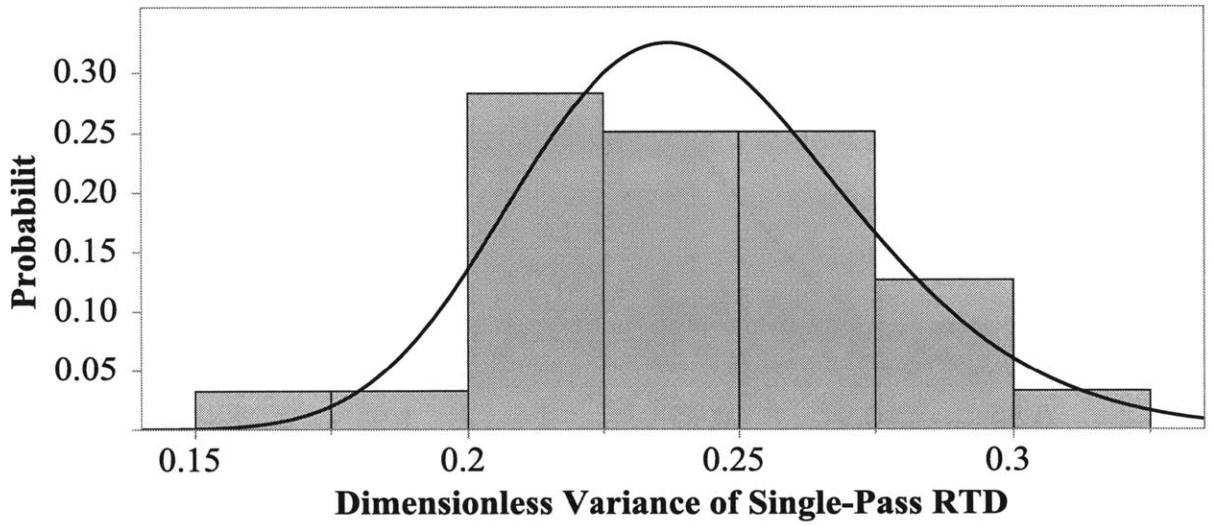

Figure 30 **Fitted Lognormal Distribution of Dimensionless Variance of Production Reactor Single-Pass RTD**

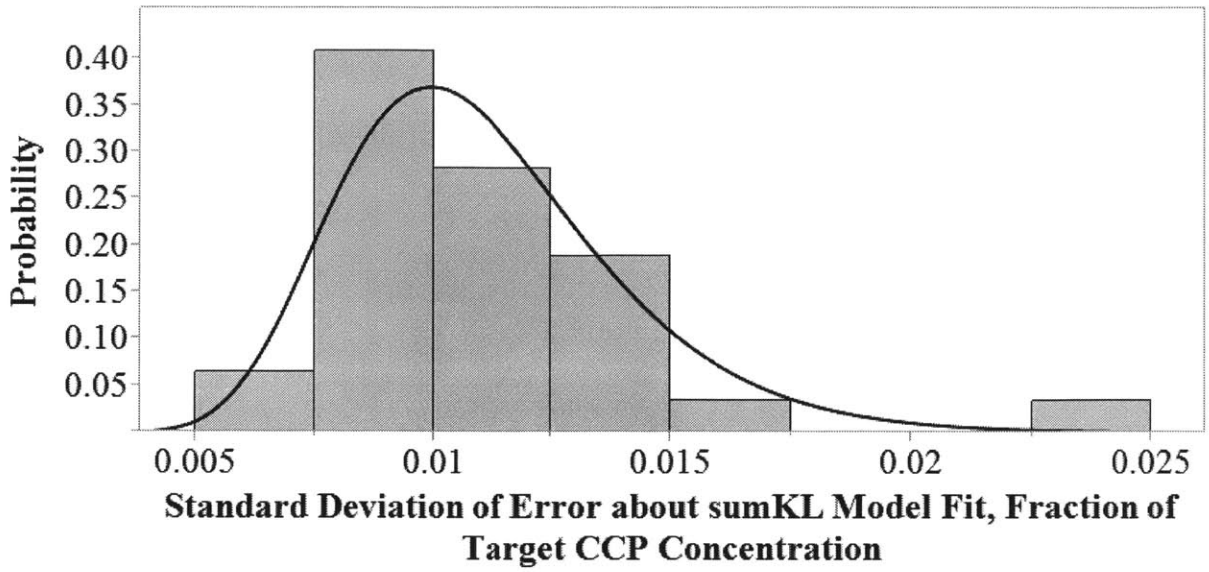

Figure 31 **Fitted Lognormal Distribution of Variability of Production Reactor CCP Concentration Measurement during Fill Operation**