Influence of Process History on intensified Design of Experiment Regression Models: A Simulation Study

Intensified design of experiment (iDoE) aims to enhance the efficiency of upstream process development by studying several input combinations within one bioreactor. The introduction of multiple stages within a process is essential to understand bioprocess dynamics, e.g., due to different growth phases within a fed-batch process. Based on iDoE, decisions can be made about whether changes in input settings can improve process performance, robustness, and product quality for the introduced stages oriented on the biological growth phases. Therefore, proper planning and analysis of iDoE are prerequisites for optimal intensified fed-batch (iFB) protocols. However, altering input settings during the bioprocess leads to varying process histories among the bioreactors, which can result in different cellular responses to the same input settings at later stages, also known as the memory effect.

Different modeling approaches can be applied to analyze iDoE data, considering the memory effect in distinct ways. The first approach is stage-wise modeling, which evaluates each stage separately while implicitly accounting for process history through the initial conditions at the beginning of each stage within a bioreactor. Alternatively, a whole process model can be built explicitly, incorporating process history during planning by including across-stage interactions. These interactions reflect the interactions between input settings at different stages, serving as proxies for the influence of process history and the memory effect. While the first approach does not consider the impact of process history during planning, the second approach necessitates a significant number of experiments to resolve the complex model structure.

This study aims to address the challenge of robust planning and evaluation of iDoEs, taking into account the influence of process history. The primary objective of this simulation study is to examine the impact of different effect sizes of process history (across-stage interactions) on our regression models based on the different modeling approaches. Simulated viable cell density (VCD) data, representing different magnitudes of process history, are utilized to benchmark the modeling approaches based on statistical criteria such as coefficient estimates (betas) and root mean square error (RMSE). Realistic effect sizes will be determined using experimental data from an iDoE where both within-stage and across-stage interactions were considered during planning. By defining cutoff criteria for critical effect sizes based on the in-silico study and real-world evidence from in vitro experiments, a strategy will be proposed to plan iDoE at the sweet spot of adequate model precision and experimental effort.

Title: Influence of Process History on Models of Biodynamics

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Short CV: Dr. Verena Nold obtained her Ph.D. at the University of Ulm in the field of molecular medicine, researching the interaction of glucocorticoids, oxidative stress, and the immune system. In June 2020, she began working at Boehringer Ingelheim Pharma GmbH & Co KG in Biberach as a PostDoc doc in the chemical manufacturing control statistics department. Since 2022, she has worked there as a senior statistician.

Summary

Multiple input factors including timing and exposure influence the biopharmaceutical upstream process, i.e., the readouts of fed-batch culture processes like cellular growth, formation of titre, and product quality. Intra-experimental input setting changes during the process enable an efficient screening of the design space by studying several input combinations with one bioreactor. In addition, this intensification of Design of Experiments (iDoE) is essential to understand bioprocess dynamics to e.g., determine whether and when changes of the input settings improve process performance, robustness, and product quality. Proper planning and analysis of iDoE therefore are prerequisites for optimal intensified fed batch (iFB) protocols. However, changes of input settings within the bioprocess and thus different process histories as well as different cellular states may cause different reactions of the cells to the same input settings later in the bioprocess (memory effect).

To account for potential memory effects during modelling, at least to approaches can be applied. One is stage-wise modelling, where the response value after each input setting change is used as additional input factor (init factor). The second approach is across-stage interaction modelling, where the model equation not only contains input combinations at the same timepoint but also interactions between input settings at different timepoints. While the first approach suffers from difficulties in planning for non-correlated init factors, the second approach requires a high number of experiments. To determine whether this many experiments are needed to obtain models that adequately describe the bioprocess dependencies and dynamics, the magnitude of prediction error of models that consider all across-stage interactions can be compared to the error of models that omit all or some of these interactions.

In a simulation study, we assess the impact of effect size of across-stage interactions on the prediction error. To this end, comparisons of the coefficient estimates (betas), the root mean squared error (RMSE), and coefficient of determination (R^2) between models with and without across-stage interactions built on viable cell density (VCD) simulations with varying effect sizes are made. Experimental data of an iDoE where all within- and across-stage interactions were considered during planning will be used to determine realistic effect sizes. Based on cut-off criteria for critical effect sizes defined in the in-silico study and the real-world evidence of the in vitro experiments, a strategy to plan iDoE at the sweet spot of adequate model precision and experimental effort is suggested.