A Demonstration of the Effectiveness of Design of Experiment Methodology for Strategic Upstream Process Development through the Completion of Transient Gene Expression Optimization and Oxygen Mass Transfer Characterization Application Studies

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BIOPROCESSING

Abstract

A thorough demonstration of holistic process understanding is the foundational goal which drives the work of many upstream process development and characterization teams. Such advanced comprehension requires viable process models that can accurately describe the effects of various process input factors on culture health, productivity, and product quality. This high level of understanding supports both process robustness and continuous improvement. Additionally, the demonstration of complete process knowledge aligns with the concept of Quality by Design (QbD), an imperative which has been emphasized heavily by the United States Food and Drug Administration (FDA).

Design of Experiment (DoE) methodology can be a valuable tool to support bioprocessing scientists and engineers in the pursuit of holistic bioprocess understanding. Strategically executed DoE studies can help teams move closer to a more complete level of process comprehension. In this work, we present two successful examples of utilizing response surface DoE designs to characterize and optimize target outputs for CHO-S upstream bioprocesses. Through these studies, we demonstrate the potential of DoE within the upstream bioprocess space for teams seeking a more advanced level of process understanding.

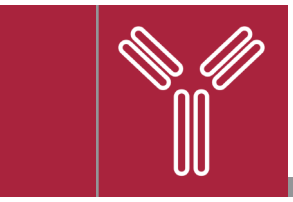
In our first study, we utilized a two-factor central composite design (CCD) to optimize both the DNA concentration and the PEI - DNA ratio within a transient gene expression (TGE) CHO-S process. Conditions were simultaneously co-optimized to maximize both post-transfection viability and transfection efficiency. Using the optimized conditions, a process was generated which predicted 70% viability and 70% transfection efficiency at 96-hours post-transfection. An independent confirmatory run was then completed with the optimized parameters. The results from the confirmatory evaluation supported the validity of the DoE-generated process model.

In the second study, a three-factor CCD was performed to characterize the effects of volume-dependent process parameters on the oxygen mass transfer within the 2-L BIOne Single-Use Bioreactor (SUB) system, manufactured by Distek, Inc. The working volume, agitation rate, and bottom air sparge rate were all evaluated as process inputs. The effects of these factors were evaluated regarding their impacts on the volumetric mass transfer coefficient of oxygen (k_La) within the system. The system understanding gained through this evaluation allowed for the effective development of a CHO-S Stir-Tank Reactor (STR) batch process. Operational definitions for all three volume-dependent process parameters could be effectively defined based upon achieving a target k_La within the bioreactor system. When evaluated in a confirmation study using ExpiCHO-S cells, highly comparable growth and viability profiles were demonstrated across both the shaker flask and the STR system models. Such effective process transfer was likely achieved due to the holistic system understanding generated through the prior execution of the DoE mass transfer characterization study.

Introduction

Historically, most bioprocesses were qualified and validated through the execution of traditional one factor at a time (OFAT) experimental analyses.[1] For these types of studies, a single experimental input factor is typically varied, while the remaining factors are controlled at constant values. This strategy can provide scientists and engineers with a direct method of demonstrating simple relationships within their processes. While the data from such studies can prove to be suitable for the completion of required process validation and drug filing documentation, the information does not necessarily provide upstream development and characterization teams with the knowledge required for true process understanding.

The limitations of OFAT methodology limit process understanding, as engineers and scientists can garner only limited knowledge regarding the interactions between the various inputs



within their bioprocesses. The lack of full understanding regarding these interactions can result in the creation of process knowledge gaps. Such gaps can make it challenging to predict how process fluctuations, deviations, and other changes across inputs would affect individual process outputs, including the critical quality attributes (CQAs) of the final drug product. This lack of understanding makes future continuous improvement projects more challenging, and more resource demanding to study and implement. Additionally, the acceptance of knowledge gaps resulting from traditional OFAT methodology is inconsistent with the overarching tenet of Quality by Design.

The concept of Quality by Design (QbD) was first described by Joseph M. Juran.[2] Juran recognized that the quality of the manufacturing process is just as significant as the quality of the final product. His ideology has been embraced by the United States Food and Drug Administration (FDA), and was emphasized in an initiative presented to bioprocessing manufacturers.[3] FDA guidance was established which described how the *quality of a therapeutic drug should ultimately be a product of the quality of the processes through which the drug was initially developed and manufactured*. It was recognized that no amount of post-production quality control analyses can increase the inherent potency, purity, or efficacy of a drug product. Rather, final drug quality must be engineered into the product during the strategic development and characterization of the manufacturing process. The effects of both critical material attributes (CMAs) and critical process parameters (CPPs) on the CQAs and other important process outputs should be fully characterized and well understood.

The fundamental concept of Quality by Design is now expected to be integrated throughout the development of next-generational therapeutic drug manufacturing processes by regulatory bodies. Alignment with this concept requires that process development and characterization teams demonstrate holistic understanding of their bioprocesses. This level of knowledge is likely beyond what is obtainable through simple OFAT-based qualification and validation studies. A demonstration of such understanding can be achieved through the execution of robust bioprocess characterization and optimization using Design of Experiment (DoE) methodology.

Design of Experiment (DoE) methods first appeared in the beginning of the twentieth century, with the work of statistician Sir Ronald Fisher.[4] Fisher's contributions revolutionized statistical analyses. In his work, Fisher encouraged a shift in mindset for his colleagues across the technical and scientific fields. He implored his peers to stop considering statistics as a tool solely for *post-hoc* experimental analyses. Rather, he encouraged that scientists and engineers consider the incorporation of such analytics into their experimental designs from the very conception of their studies.[5] With this transition, DoE began emerging as a means of process improvement across both the agricultural and chemical industries.[6] The presence of DoE within the pharmaceutical space is recognized to have originated with the work of Marlow and Shangraw, during their characterization of the dissolution of sodium salicylate.[7] In the decades following, DoE has continued to gain relevance within the pharmaceutical space. More than 800 references had been recognized on the subject matter since 2016.[8]

The objective of DoE is to utilize a strategically defined design space to support the characterization of the relationships between process input factors and output responses. Unlike the previously described OFAT analyses, when using DoE, multiple factors can be analyzed simultaneously within the design space. This characteristic of DoE allows for elucidation of the interactions between multiple factors within the process. Additionally, data from a properly executed DoE study can describe the levels of influence of the input factors on the process responses. Using DoE analyses, the magnitude of how individual factors impact specific process outputs can be well characterized and described.

From a resource perspective, DoE holds another advantage as compared to OFAT methodology. Using DoE, the number of studies and experimental replicates can be reduced significantly in comparison to the number of trials required to test each factor independently across multiple OFAT experimental iterations. With the high material costs associated with the consumables necessary for cell culture studies, this advantage can be highly significant for many laboratory teams.

Three key elements of a DoE study are replication, randomization, and blocking.[9] Replication allows for experimental noise to be analyzed and quantified. Randomization is critical for the proper control of hidden or unknown variables during the experimental process (i.e. time of day, identity of analyst, etc.). Blocking is used when experimental challenges make true randomization unfeasible. Blocking involves first dividing the study conditions into separate groups. These groups are then evaluated during multiple experimental iterations, with treatments randomly allocated within each block.

Within the biopharmaceutical space, the most common DoE methods utilized are screening designs and response surface designs.[7] Screening designs are selected when historical data are not available to provide insight regarding the most influential input factors on identified process outputs. For such projects, screening designs can be utilized to provide process scientists and engineers with the data necessary to identify key factors as highly impactful.[10] Impactful factors will those most suitable for subsequent characterization and optimization. Examples of DoE screening include two-level full factorial designs, fractionate factorial designs, and Placket-Burnam designs. These types of experiments will support the simultaneous screening of large numbers of process inputs in a highly efficient, cost-effective manner.

Further characterization and optimization of key influential process input factors can be performed using response surface designs. The results of response surface designs describe the curvature of the process outputs in response to fluctuations across factor inputs. This level of understanding supports robust process characterization and process parameter optimization efforts. For such studies, central composite designs (CCD) or Box-Behnken designs are often utilized.

In this work, we performed two process optimization and characterization studies using response surface designs. During the first study, we used a two-factor CCD to define optimal operational definitions for DNA concentration (µg / mL) and PEI – DNA ratio (w/w) process parameters within an ExpiCHO-S green fluorescent protein (GFP) transient gene expression process. Two target output responses were simultaneously co-optimized: transfection efficiency and post-transfection viability. The DoE model demonstrated that process outputs of 70% transfection efficiency and 70% post-transfection viability were possible under optimized conditions. The model predicted optimized response was then verified during a subsequent confirmation evaluation.

The second study involved the characterization of the effects of volume-dependent process parameters on the overall oxygen mass transfer within the 2-L BIOne Single Use Bioreactor (SUB) system. Input factors for this study were working volume (mL), agitation (rpm), and bottom air sparge rate (sccm). The measured output response was the volumetric oxygen mass transfer coefficient (k_La). Characterization data from the DoE analysis was used to generate suitable volume dependent process parameter operational definitions for a ExpiCHO-S batch process. A subsequent process confirmatory evaluation using these operational definitions demonstrated cell growth and viability trends which were highly comparable to shaker-flask model controls. Results from both studies demonstrate the suitability of DoE analyses for process characterization and optimization within the upstream bioprocessing space.

Study 1: Characterization and Optimization of a Transient Gene Expression ExpiCHO-S Process with a Two-Factor Central Composite Design

Transient Gene Expression (TGE) has emerged as a powerful bioprocessing tool that has demonstrated utility across a variety of upstream applications. TGE offers laboratory teams considerable time and resource savings when compared to the process of stable monoclonal cell line generation. Such cell models can take years to successfully engineer, validate, and cryopreserve. TGE techniques can help laboratories decrease these timelines considerably. Additionally, for applications where monoclonal populations are not required, TGE offers bioprocessing scientists and engineers a viable option for effective and efficient material generation.

One of the most common nonviral methods of TGE involves the use of 25 kDa linear polyethylenimine (PEI). The PEI molecule is an organic cation polymer which has been demonstrated to effectively form complexes with negatively charged DNA molecules.[11] The final charges of these complexes are net positive. This positive charge allows the complexes to bind to the negatively charged surfaces of mammalian cells. This binding then facilitates the subsequent endocytosis of the DNA – PEI complexes.[12] This technique has proven to be a highly cost-effective means of TGE for many laboratories.

Previous work has demonstrated that both the DNA concentration and the PEI - DNA ratio used the during transient gene expression transfection processes are highly relevant process parameters.[13] To maximize transfection efficiency, the PEI – DNA ratio must be sufficiently high enough to support the effective formation and endocytosis of the PEI - DNA complexes. However, it has also been demonstrated that PEI has the potential to be cytotoxic, so excessively high concentrations can become detrimental to culture health and productivity.[14] Therefore, DNA and PEI concentrations within the transfection process must be strategically defined to balance both transfection efficiency and post-transfection viability. A response surface DoE provides a means to simultaneously optimize the transfection processes parameters for both process outputs.

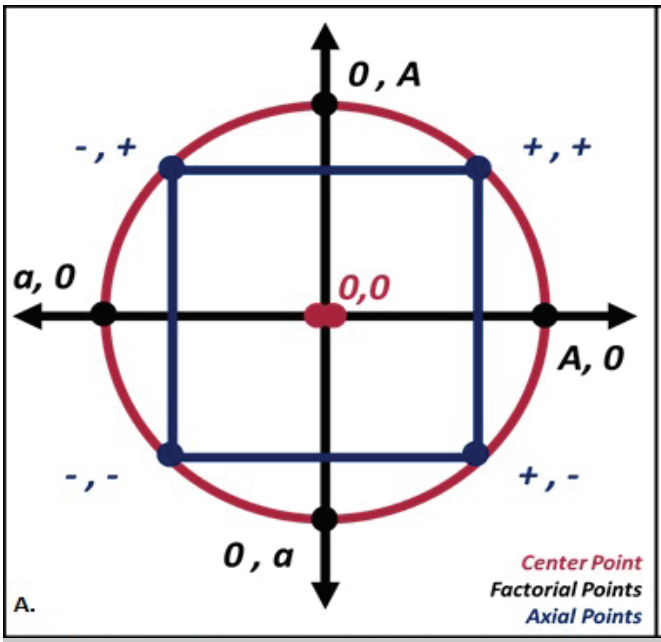
To demonstrate the suitability of DoE for TGE optimization, a two-factor CCD was executed for the optimization of both the DNA concentration and the PEI – DNA ratio within an ExpiCHO-S GFP transfection process. Process output responses for this study were transfection efficiency and post-transfection viability, each measured at 96-hours post-transfection. The design space for the DoE is shown in **Figure 1**.

The cell model used during the evaluation was the ExpiCHO-S cell line (Gibco, A29127). The cells were thawed into FreeStyle™ CHO Expression Medium (Gibco, 12651022) which had been supplemented with 0.1% Pluronic F-68 (Gibco, 24040032). The study was performed within 125-mL baffled shaker flasks using a 15 mL final working volume. Cells were passaged three times out-of-thaw (OOT) prior to transfection seeding. Subculturing was performed between 72 and 96 hours, when the culture viable cell densities (VCD) reached approximately 3.0 – 4.0 x 10^6 cells per mL. During passaging, cells were reseeded at a target VCD of 0.35 x 10^6 cells per mL in fresh basal medium.

On the day prior to culture transfection, cells were seeded at a VCD of 1.0 x 10^6 cells per mL. Pre-transfection incubation conditions were 37°C, 5 % CO_2, and atmospheric O_2. Culture suspension was maintained using a CO-Z Orbital Shaker operated at 110 rpm.

During the transfection process, PEI – DNA complex formation was performed in a total of 2 mL of solution. Prior to complex formation, working stock solutions of both PEI and DNA were prepared in culture basal medium. Stock solution concentrations were 100 µg / mL (DNA) and 200 µg / mL (PEI). Stock solution tubes were briefly vortexed prior to the complex formation step.

During the complex formation step, the appropriate volumes of both working solutions were combined in 15 mL conical tubes, in which the necessary volumes of basal medium had already been aliquoted. Complex formation tubes were inverted and gently vortexed on a low setting to homogenize the solutions. Post-homogenization, the tubes were incubated for five-minute at room temperature. Upon completion of the incubation period, the complex solutions were added to culture shaker flasks. After the complex addition was complete, all shaker flasks were immediately returned to the culture incubator. The temperature of the incubator was then decreased to 32°C. No other incubation parameters were changed post-transfection.



B.

Pattern	DNA Concentration (µg / mL)	PEI : DNA (w / w)
0A	2.25	5.00
0a	2.25	3.50
++	3.00	5.00
--	1.50	3.50
+-	3.00	3.50
A0	3.00	4.25
-+	1.50	5.00
00	2.25	4.25
A0	1.50	4.25
00	2.25	4.25

Figure 1: A two-factor central composite design (CCD) DoE was selected for the initial response surface design of experiment (DoE) optimization for the ExpiCHO transient gene expression process. (A.) Visualization of a two-factor CCD. **(B.)** Overview of the design space evaluated during the transient gene expression process optimization project.

During culture analyses, cell viability was determined with propidium iodine staining (Bio-Rad, 1351101). Both viability and GFP expression were measured using flow cytometry (Accuri C6, BD Biosciences). Pre-transfection baseline analyses were preformed four hours prior to transfection. Post-transfection analyses were performed every 24 hours after transfection. Analyses were completed through 96 hours post-transfection. Transfection data were analyzed using JMP15 (SAS) software.[15]

The process model generated from the response surface DoE was demonstrated to be highly suitable to describe the transfection process. As shown in **Figure 2**, the correlation coefficient values for predicting both transfection efficiency (R^2 = 0.98) and post-transfection viability (R^2 = 0.97) demonstrate a strong relationship between the input factors and output responses. Additionally, the p-values for both transfection efficiency (p-value = 0.0020) and post-transfection viability (p-value = 0.0038) show the significance of the models and demonstrate their suitability to accurately describe the transfection process. The residual by predicted plots also support the model validity, as both plots demonstrate random distributions and the absence of any visible trends.

Transfection conditions suitable for co-optimization of both post-transfection viability and transfection efficiency were determined using the surface plot and prediction profiler features of the JMP15 software. As shown in **Figure 3**, process inputs of 2.1 µg / mL DNA and a PEI – DNA ratio of 3.75:1 (w/w) were predicted to yield approximately 70% transfection efficiency and 70% post-transfection viability at 96-hours post-transfection. These values were determined to be the optimal process parameter definitions for the co-optimization of both process output responses.

As a final assessment of the TGE model validity, a confirmation transfection evaluation was performed. For the confirmation study, the cell culture methods and transfection process were executed as previously described. The results of the confirmation study are shown in **Figure 4** and **Figure 5**. These results demonstrate the accuracy of the model for predicting both transfection efficiency and post-transfection viability for the ExpiCHO-S GFP TGE process. Overall, the results of this study support the suitability of utilizing response surface DoE methods for the characterization and optimization of TGE bioprocess applications.

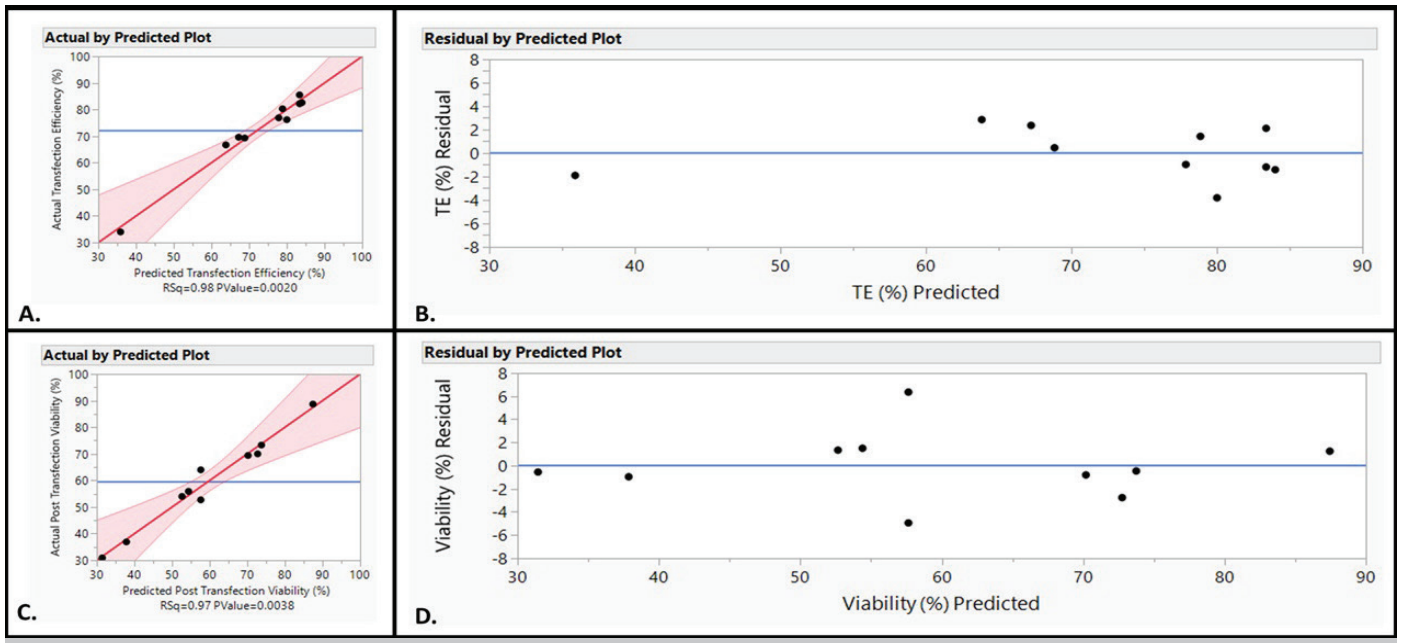


Figure 2: Results from the response surface DoE optimization demonstrate significance of models to predict both transfection efficiency and viability 96-hours post-transfection. Data shown: actual by predicted plot and residual plot for transfection efficiency model **(A. & B.)** and actual by predicted plot and residual plot for post-transfection viability model **(C. & D.)**

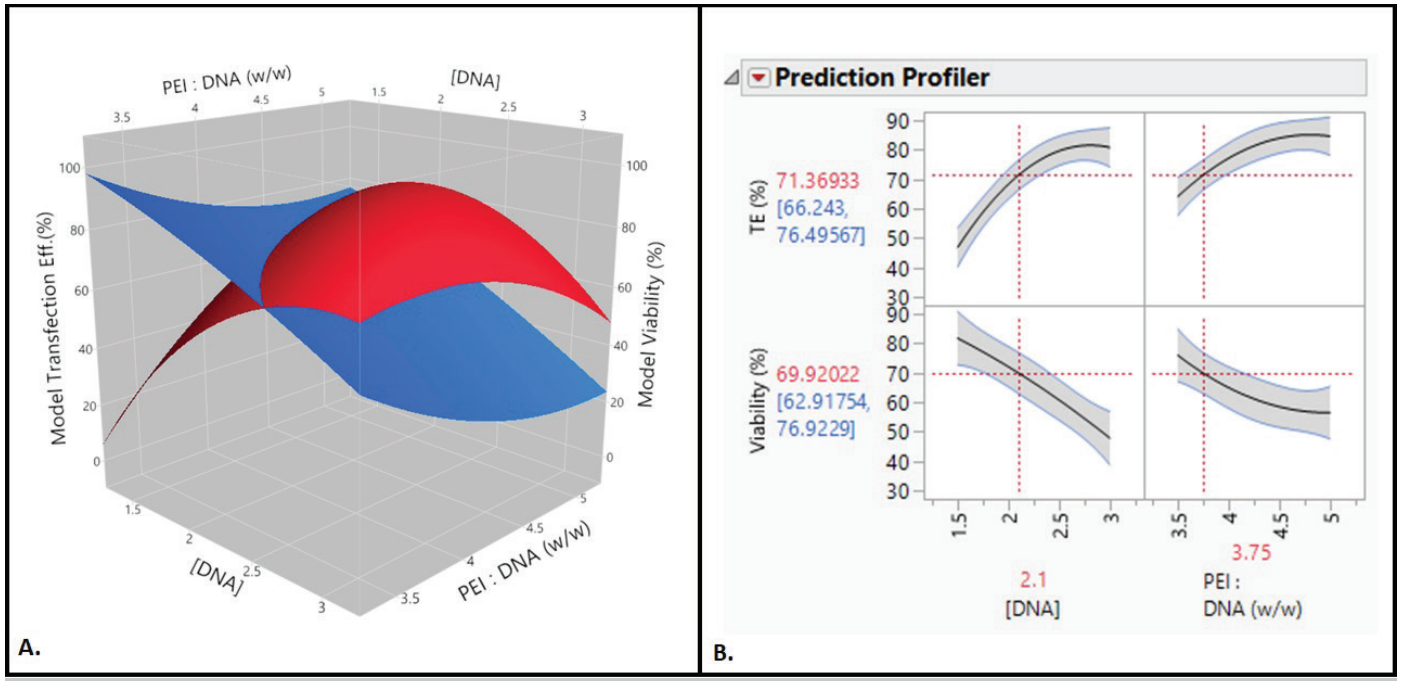


Figure 3: Optimization of both viability and transfection efficiency using response-surface DoE modeling (96-hours post-transfection). (A.) Surface plot and **(B.)** Prediction Profile both suggest that transfection efficiency and viability can be simultaneously optimized to approximately 70% using DNA concentration of 2.1 (µg / mL) and a PEI:DNA ratio of 3.75:1 (w/w).

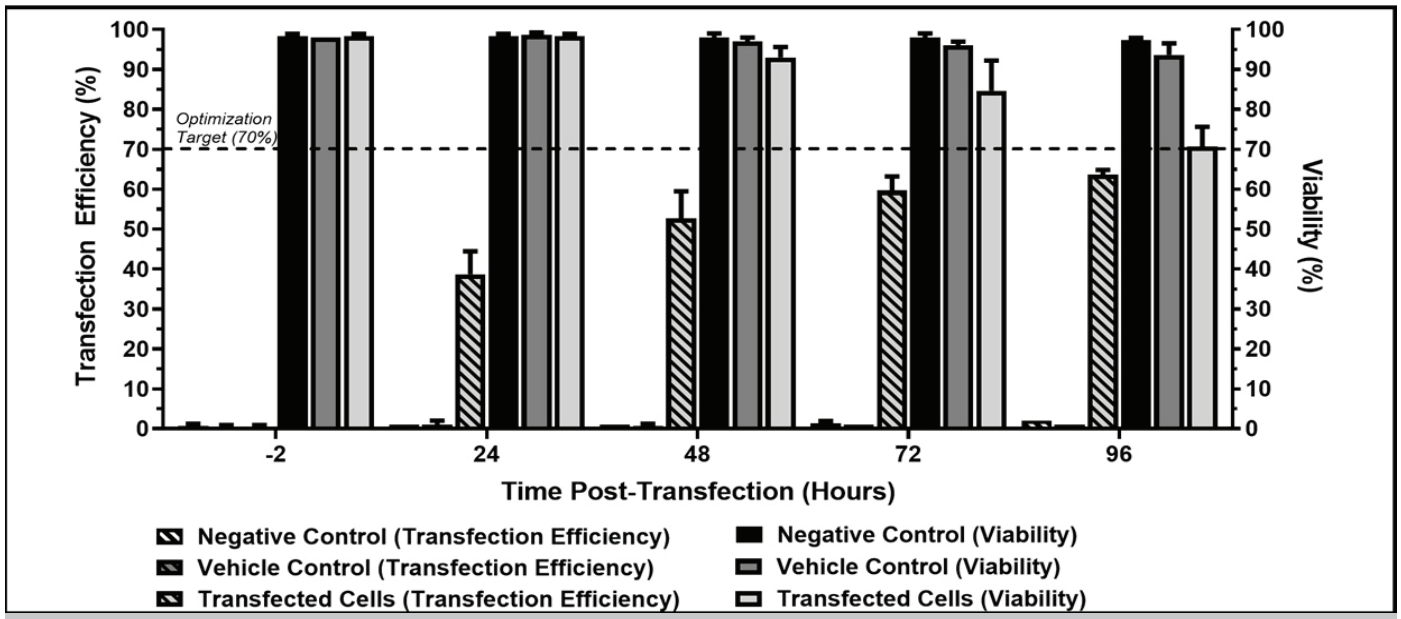


Figure 4: Results from transfection parameter confirmatory study demonstrate the suitability of response surface DoE modeling for optimization of a transient gene expression upstream bioprocess. Optimized parameters were defined to target 70% viability and 70% transfection efficiently at 96-hours post-transfection. Both cell viability and transfection efficiency were determined using flow cytometry.

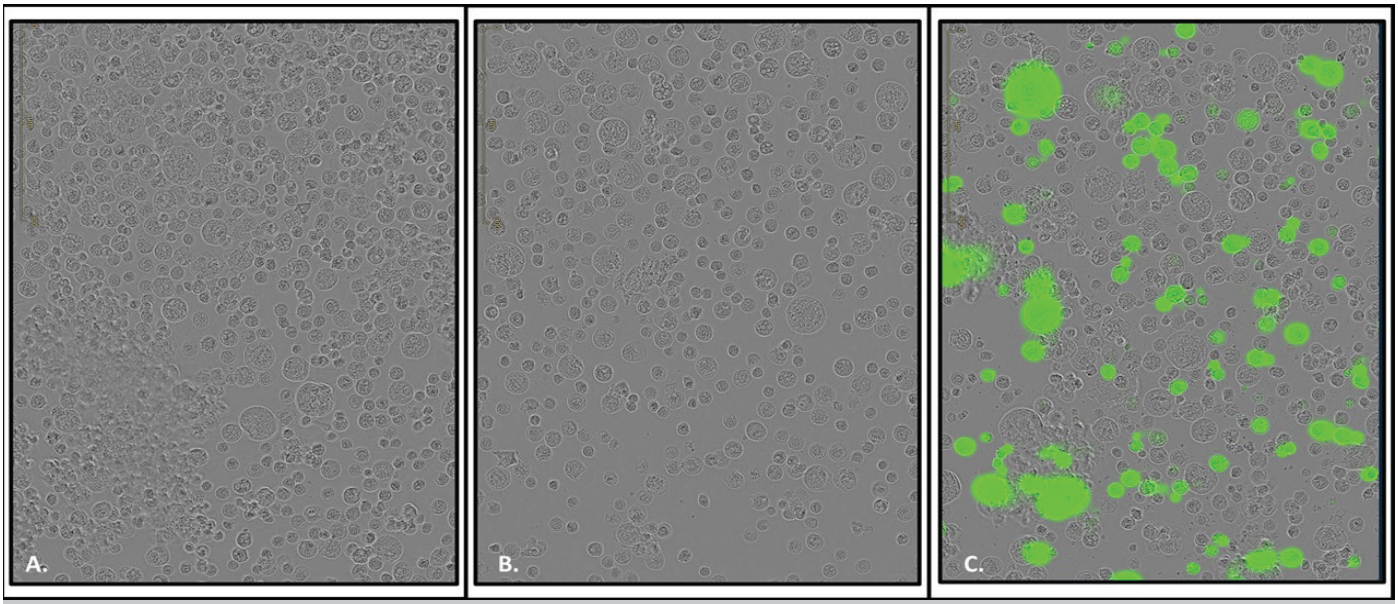


Figure 5: 96-hours post-transfection fluorescent microscopy images of (A.) non-transfected cells, (B.) PEI vehicle control cells, and (C.) GFP-transfected cells. Images support transfection efficiency data and viability data obtained from flow cytometry analyses. Cells were transfected using optimized conditions that were identified during response surface DoE optimization study: DNA concentration of 2.1 µg / mL and a DNA : PEI ratio of 1 : 3.75 (w/w). All images at 20x magnification.

Study 2: Characterization of the Effects of Volume-Dependent Process Parameters on Oxygen Mass Transfer within the 2-L BIOne Single Use Bioreactor System and the Strategic Development of an ExpiCHO-S Batch Process using a k_La Central Criterion

Upstream bioprocesses are composed of both volume dependent and volume independent process parameters.[16] Operational definitions for volume independent process parameters will be held constant across different bioreactor systems, regardless of bioreactor geometry or volumetric scale. Volume-independent process parameters include input factors such as pH setpoint, glucose concentration, and target post-inoculation viable cell density.

In contrast to volume independent process parameters, the operational definitions for volume dependent process parameters will change as bioprocesses are transferred across various scales and bioreactor systems. Volume dependent parameters include input factors such as agitation rate, vessel working volume, and sparged air flow rate. When transferring upstream bioprocesses to different systems, operational definitions for volume dependent parameters are typically engineered using linear scaling strategies. Such strategies are based upon the conservation of a central criterion across all scaling platforms.[17,18] The engineering of suitable volume dependent process parameters can be one of the most challenging aspects of process development or process transfer projects.

Volume dependent parameters are the primary factors which determine the overall oxygen transfer rate (OTR) within a bioreactor system. The OTR describes the frequency at which molecular oxygen dissolves from the gaseous state to the aqueous state within a bioreactor system.[19] The aerobic requirements of mammalian cell culture necessitate that the oxygen transfer rate within the system be sufficient meet the demands of cellular metabolic processes. Insufficient oxygen transfer within the system can have adverse effects on cell health, productivity, and product quality.[20,21,22] However, if volume dependent operational parameters are defined too aggressively, the resulting hydrodynamic shear forces generated within the process may become detrimental to the health of the culture.[23] As such, the engineering of suitable operational definitions for volume dependent process parameters is critical for upstream bioprocesses. For optimal performance, a balance between oxygen transfer and shear forces must be demonstrated within the system. This balance can be achieved through strategic scaling based upon the conservation of an appropriate central criterion.

Functionally, the OTR within a bioreactor can be defined as the product of the volumetric oxygen mass transfer coefficient (k_La) and the oxygen concentration gradient ($C^* - C_L$) within the system.[24] This relationship is shown in **Equation 1**. The oxygen concentration gradient will be dependent on the definition of the dissolved oxygen process parameter setpoint, which is typically maintained between 30% - 50% across most mammalian cell culture processes. As the DO setpoint is a volume independent parameter, it will likely be held constant across process scales during development. If the system k_La is also held constant, the OTR should be consistent across all scales and systems during process transfer operations. The direct relationship between k_La and OTR within bioreactor systems makes k_La a suitable candidate to serve as a central scaling criterion for upstream bioprocess transfers.

$$OTR = (C^* - C_L) \times (k_La) \quad \textbf{(Eq. 1)}$$

The system k_La is functionally the product of both the liquid phase mass transfer coefficient (k_L) and the gas-liquid interfacial surface area (a).[25] For simple batch processes, previous work demonstrates how bioreactor systems whose defined operational process parameters result in system k_La values between 2.5 hr^{-1} to 5.0 hr^{-1} are likely suitable to support the expansion of most mammalian cell cultures.[26,27] Processes whose defined operational parameters result in system a k_La in this range typically can meet the aerobic requirements of cultures, without the causing excessive levels of hydrodynamic shear forces to be generated within the systems.[28] Advancements in cell line development have supported the creation of high-density cell lines with peak VCDs on the orders of 20 – 80 x 10^6 cells per mL.[29,30] These cells are typically cultured within fed-batch or perfusion processes. Due to the higher peak cell densities of these processes, higher overall system k_La values may be targeted for these specific applications.

To achieve a target k_La within a bioreactor process, the system must be characterized in a manner where the effects of the volume dependent process parameters on the mass transfer of oxygen within the system are well understood. A response surface DoE is highly appropriate for use in such characterization efforts. By analyzing the effects of agitation rate, bottom air sparge rate, and working volume on the system k_La, one can accurately describe the effects of the volume dependent process parameters on the overall oxygen transfer rate within the bioreactor system.

The BIOne Single-Use Bioreactor (SUB) is a benchtop Stir-Tank Reactor (STR) manufactured by Distek, Inc. The system was strategically designed to improve scale-down model (SDM) experimental process flows through a considerable reduction in system preparation labor hour requirements, as shown in **Figure 6**. The liner composition of the bioreactor also has the potential to facilitate accelerated process scale-up for disposable manufacturing processes, due to contact surface homogeneity between development and production systems. The integration of a BIOne SUB as a SDM for a disposable production process allows for a liner-based contact layer to be conserved across both the benchtop and production scales.

To support upstream bioprocess transfers into the BIOne SUB system, a k_La characterization project was performed on BIOne SUB model 2022-1002 (2-L volume, single pitch blade impeller, and 7 × 1.5 mm drilled hole sparger). The goal of this project was to support the strategic engineering of operational definitions for volume dependent process parameters suitable for mammalian cell culture batch processes. A response surface three-factor CCD was identified as suitable for use for the k_La characterization project.[31] The design space for the three-factor CCD was defined based upon the current operational limits of the 2-L BIOne SUB system. The design space for the study is shown in **Figure 7**.

During the study, system k_La was experimentally determined using the static gassing out method, as described in de Ory, Romero, and Cantero, 1999.[32] The evaluation was performed using a model medium which utilized common cell culture media components at typical upstream bioprocess concentrations. The composition of the model medium was 1.6 g/L sodium bicarbonate, 7.6 g/L sodium chloride, and 0.1% Pluronic F-68 (Gibco, 24040032). Previous work has demonstrated the suitability of the use of such a model medium for the broad application of mass transfer evaluations.[33] All data generated during the characterization project were analyzed using JMP15 (SAS) software.[15]

As shown in **Figure 8**, the resulting model generated from the three factor CCD was determined to be suitable for describing the oxygen transfer within the 2-L BIOne SUB system. The model demonstrated strong correlation ($R^2 = 0.99$) and a high level of significance (p-value < 0.0001). Model residuals displayed randomness, with no indication of a visual pattern or trend. The effect summary data also demonstrated that the input factors of working volume and agitation were primarily responsible for driving oxygen transfer within the system. The visual curvature of the surface plots, shown in **Figure 9**, supported the results of the effect summary.

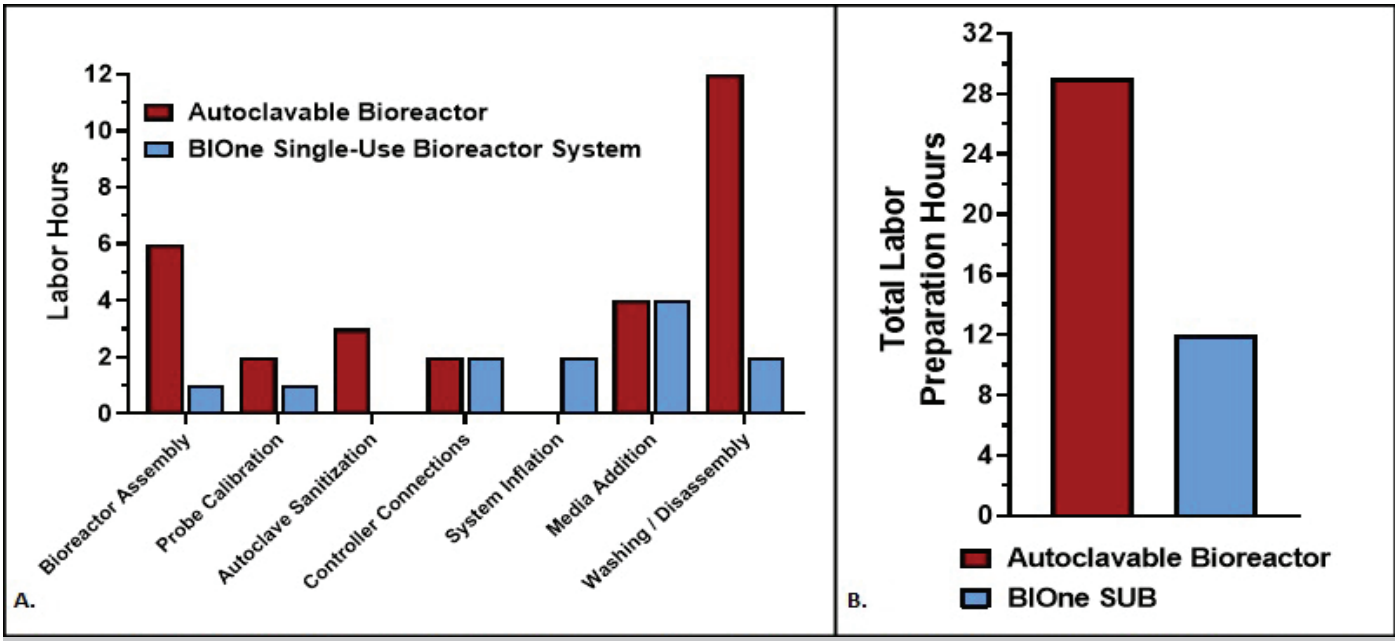
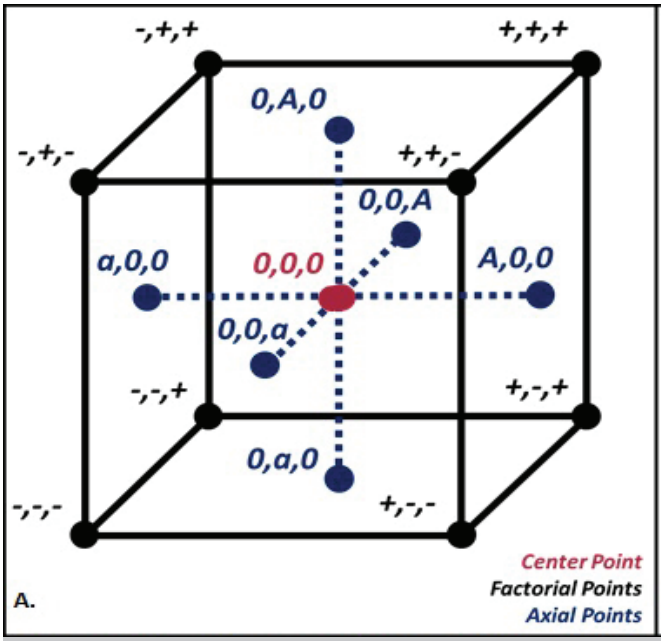


Figure 6: (A) Individual task hours and (B) overall labor estimates for the preparation and disassembly of BIOne System versus traditional autoclavable glass bioreactors. Integration of BIOne SUB system into process demonstrates the potential to decrease labor hours by over 50%. Data are estimates based on labor requirements to execute a n = 12 bioreactor study.



Pattern	Vessel Volume (mL)	Sparged Air Flow (sccm)	Agitation Rate (rpm)
00A	1450	83	400
−−−	900	15	100
+−−	2000	15	100
++−	2000	150	100
0A0	1450	150	250
−+−	900	150	100
+++	2000	150	400
A00	2000	83	250
−++	900	150	400
−−+	900	15	400
000	1450	83	250
a00	900	83	250
0a0	1450	15	250
00a	1450	83	100
000	1450	83	250
+−+	2000	15	400

B.

Figure 7: A three-factor central composite design (CCD) DoE was selected for mass transfer characterization modeling of the 2-L drilled-hole sparger BIOne Single Use Bioreactor. (A.) Visualization of a three-factor CCD. **(B.)** Summary of the design space evaluated during the oxygen mass transfer characterization project.

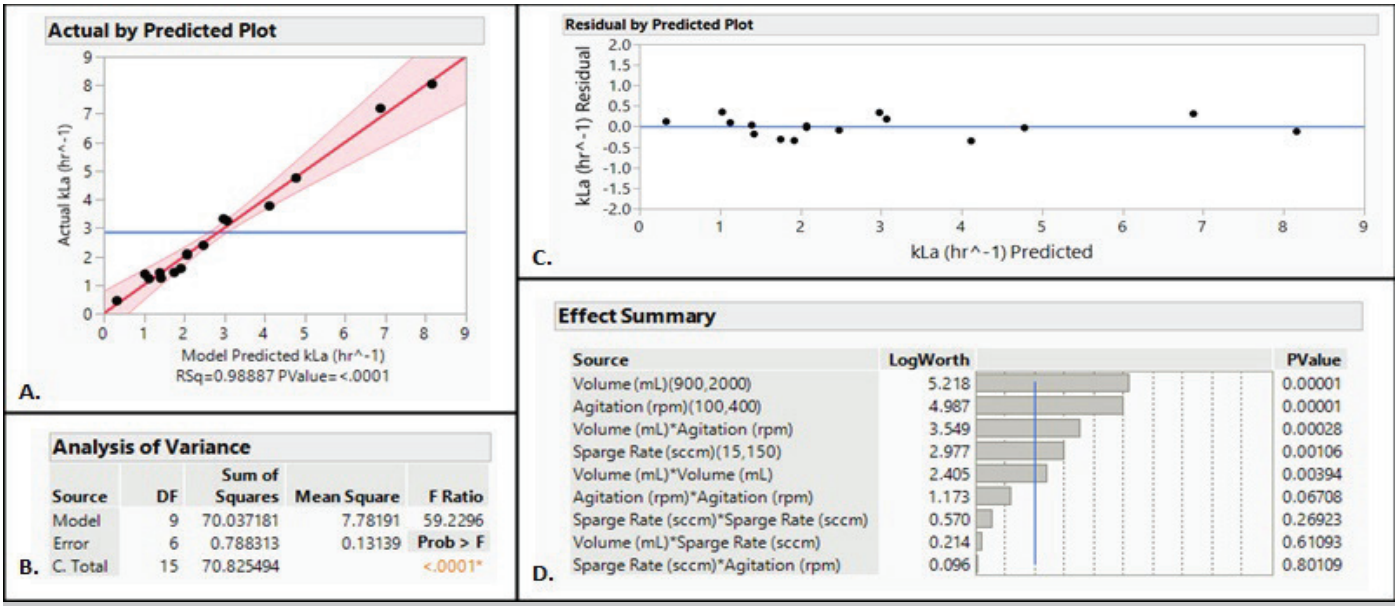


Figure 8: (A.) Actual by predicted plot, (B.) Analysis of Variance, and (C.) Residual Plot from mass transfer characterization study demonstrate the suitability of the model to describe oxygen transfer within the BIOne Single Use Bioreactor (SUB). The model demonstrates strong correlation and high significance. **(D.) Effects Summary** data suggests that agitation rate and working volume inputs have the greatest impact on overall oxygen mass transfer within the bioreactor system.

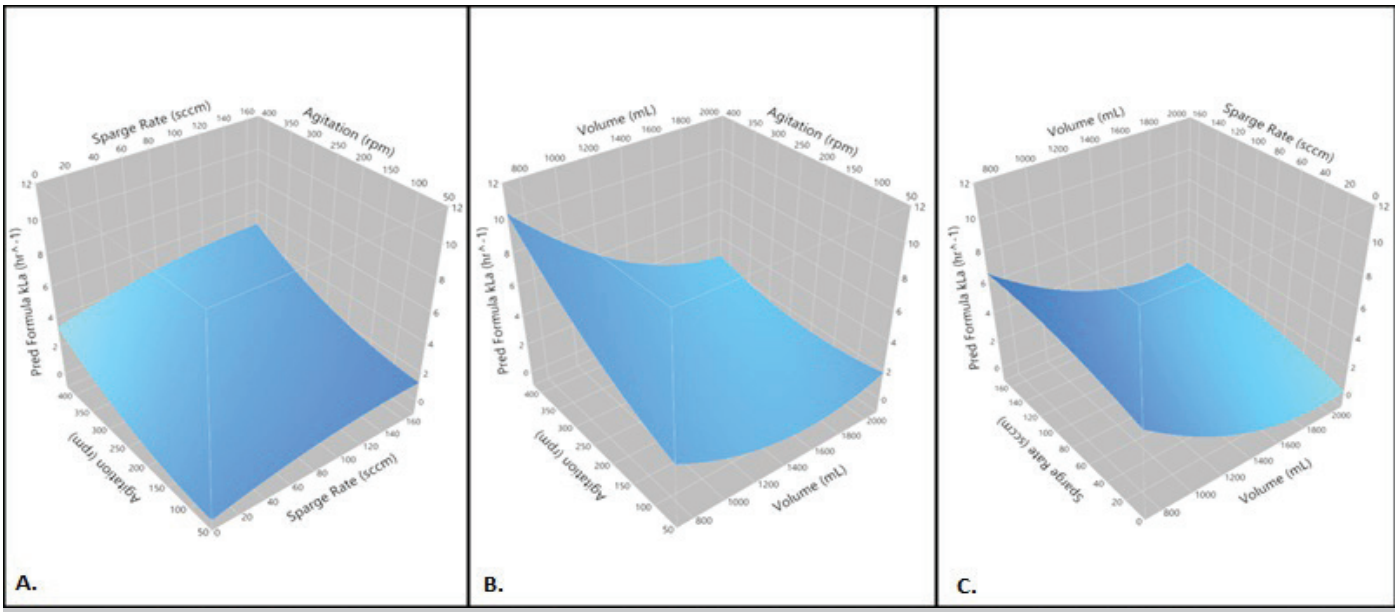


Figure 9: The curvature of the surface plot models demonstrate that the process inputs of agitation rate and working volume have the strongest influence on the overall oxygen mass transfer rate in the system. Curvature of the models supports the statistical data generated from the effects test. Surface plots shown include: **(A.)** Agitation and Sparge Rate, **(B.)** Agitation and Volume, and **(C.)** Sparge Rate and Volume.

Using the BIOne SUB mass transfer model, a set of volume dependent operational parameter definitions were created for a novel mammalian cell culture batch process. Definitions were engineered based upon a target bioreactor system k_La value between 2.5 – 3.0 hr^{-1}. Using the isosurface model and prediction profile features of the JMP15 software, operational setpoints of 300 rpm (agitation rate), 1400 mL (working volume), and 60 sccm (bottom air sparge rate) were defined for the process, as shown in **Figure 10**. Power input for the defined process parameters was 35 W/m³. Volumetric air flow rate was 0.04 vvm.

To confirm the suitability of the volume dependent operational parameter definitions for a mammalian cell culture batch process, ExpiCHO-S cells (Gibco, A29127) were cultured within the BIOne SUB system. The cells were thawed into FreeStyle™ CHO Expression Medium (Gibco, 12651022) which was supplemented with 0.1% Pluronic F-68 (Gibco, 24040032). Cells were expanded OOT within 250 mL baffled orbital shaker flasks. Cells were passaged three times prior to bioreactor inoculation. Subculturing was performed as previously described within the TGE characterization and optimization study.

Bioreactor volume-independent process parameters were operationally defined at the following setpoints: pH = 7.2 (deadband of 0.20), dissolved oxygen = 40%, temperature = 36.5°C. A solution of 1M sodium bicarbonate was used to maintain pH in combination with carbon dioxide sparging. The bioreactor was inoculated at a target VCD of 0.35 x 10^6 cells per mL. All viability and cell count analyses were performed with trypan blue exclusion staining (Countess II, Applied Biosystems).

Results from the ExpiCHO-S process are summarized in **Figure 11**. Culture growth and viability trends from the BIOne SUB demonstrate comparability to trends observed within the 250-mL shaker flask controls. These data suggest that the operational definitions for the volume dependent process parameters were suitable to meet the aerobic requirements of the culture without producing excessive levels of hydrodynamic shear forces within the system. The observed comparability between models supports that DoE-based mass transfer analysis can be an effective means of characterizing a bioreactor system for process transfer and scaling projects.

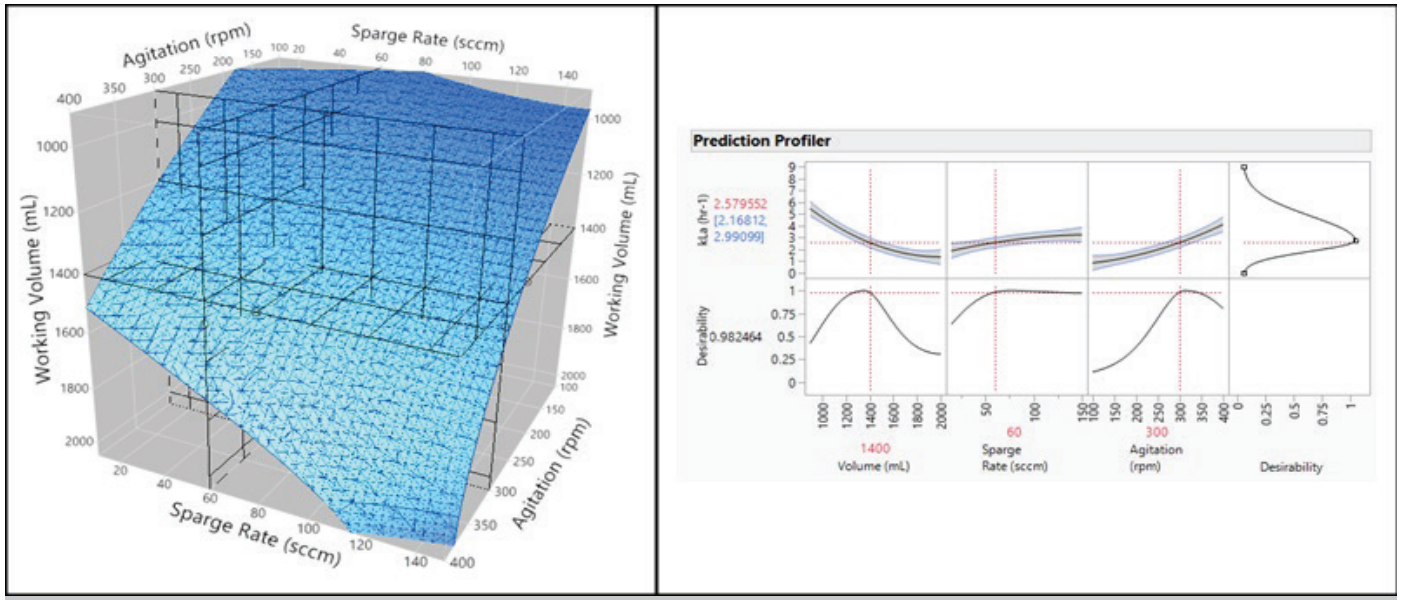


Figure 10: Predictive modeling using system characterization data allows for the identification of volume-dependent process parameter definitions which will be suitable to support an ExpiCHO batch upstream process. Both the **(A.) Isosurface Model** and the **(B.) Prediction Profiler** results demonstrate that the selected process parameter definitions (1400 mL, 300 rpm, 60 sccm) will result in sufficient oxygen transfer to support the aerobic requirements of the culture (target k_La = 2.5 - 3.0 hrs-1).

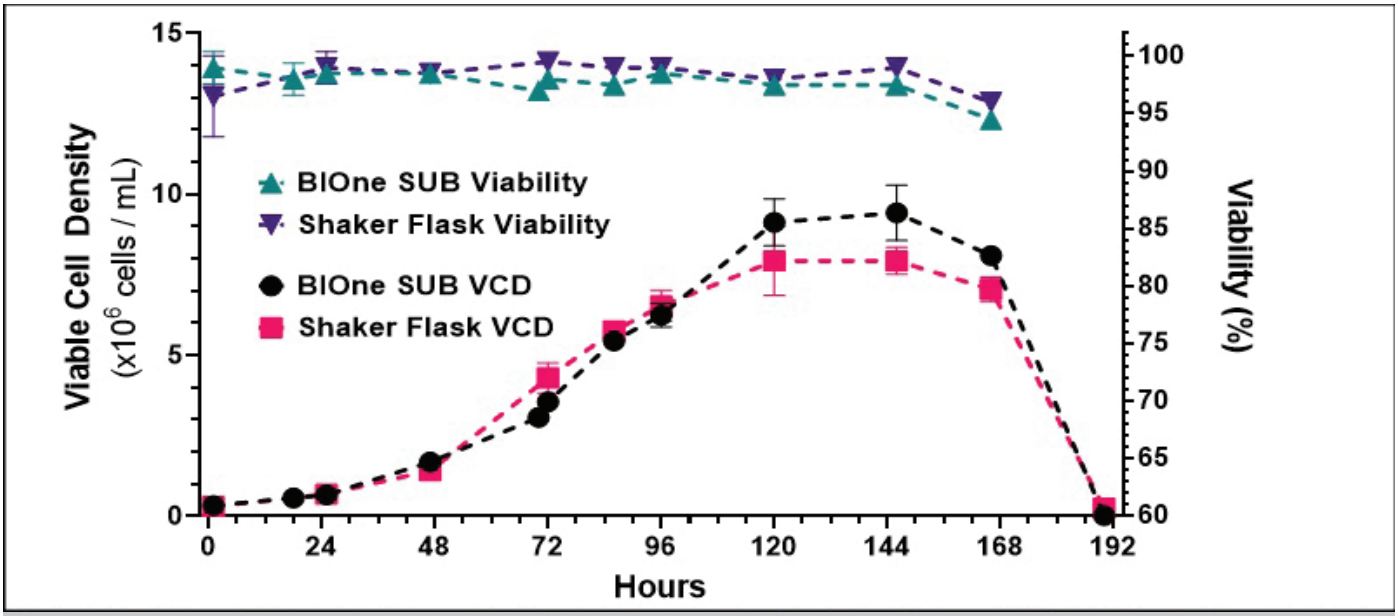


Figure 11: Offline cell health data demonstrates effective process scaling from a 250 mL shaker flask model to a 2-L BIOne Single Use Bioreactor (SUB) system. Operational definitions for agitation (300 rpm), working volume (1400 mL), and bottom air sparge rate (60 sccm) were determined using oxygen mass transfer characterization model. Parameter definitions were selected to target an overall bioreactor system k_La of 2.5 - 3.0 hrs-1.

Conclusions

Design of Experiment (DoE) methodology can be a powerful tool for upstream bioprocess optimization and characterization teams. As multiple input factors can be evaluated simultaneously, such an approach offers the potential for higher level process understanding. In this regard, the integration of DoE experimental methods aligns closely with the FDA's guidance regarding the importance of Quality by Design within the biopharmaceutical space.

In this work, we demonstrated the suitability of response surface DoE designs for both the optimization of a transient gene expression process and the characterization of oxygen mass transfer within a 2-L BIOne SUB system. As previously described, both efforts yielded positive results. Co-optimization of both post-transfection viability and transfection efficiency was demonstrated for the TGE process. Optimal process parameters definitions were then verified with a confirmatory transfection evaluation. The mass transfer characterization of the BIOne SUB facilitated the engineering of suitable process parameters definitions for a mammalian cell culture batch process. This suitability was then demonstrated through the execution of a confirmatory study which involved the expansion of ExpiCHO-S cells within both 2-L BIOne SUB and 250-mL orbital shaker flask systems.

Integration of DoE based methods into upstream bioprocess characterization offers a tremendous amount of potential to advance holistic process understanding for process development teams. The inclusion of such types of experimental designs can maximize the utility of data generated from a fixed set of resources. Process teams should strongly consider DoE implementation within their experimental designs as they plan their next characterization or optimization studies.

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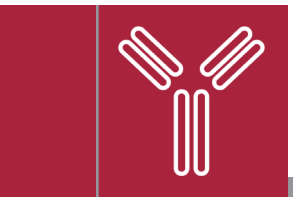

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