Study on Interactions between Fed-Batch and Batch Operating Parameters for the Development of Monoclonal Antibody Fed-Batch using Design of Experiments

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Abstract

In conventional fed-batch process development approaches, batch operating parameters (such as pH, temperature, seeding density, dissolved oxygen concentration) are kept constant and only feeding parameters such as feeding time, post-feed concentration are manipulated. The batch and fed-batch operating parameters are assumed to be independent of each other. This approach to process development ignores any interactions that might exist between the batch and fed-batch operating parameters and therefore are not evaluated. However, in a complex bioprocess, none of the factors affecting the process can be assumed to be independent of each other and mutually exclusive. Therefore, in this study an attempt was made to study the interaction between fed-batch operating parameter-post feed glucose concentration (A) and batch operating parameter-seeding density (B), temperature (C), and dissolved oxygen concentration (D) by their simultaneous manipulation, as well as the effect of these interactions on cell growth and monoclonal antibody (mAb) production. NS0 cell line producing the mAb’s against carcino-embryogenic antigen (Anti-CEA) was used. The final mAb concentration, viable cell density and integral of viable cell concentration (IVCC) were the responses evaluated. Statistical analysis experimental data showed that parameter (A) and its interaction with parameter (B) were the main factors affecting both the response variables. In comparison to the batch run which yielded 5.21 mg/L mAb, the developed fed batch process increased the mAb titer by 10 fold (59.40 mg/mL), and the IVCC was increased by 7 fold. The maximum VCD value (3.46×10^5 cells/mL) of the developed fed batch process was 1.25 over fold the value for batch.

Keywords: Fed-batch; Design of experiments; Monoclonal antibody; Anti-CEA; NS0

Introduction

Following the pioneering development of hybridoma technology by Kohler and Milstein in 1975, the usage of mAbs as therapeutic and diagnostic agents has seen a rapid increase during the past decade. There are several approved mAb based therapeutic and diagnostic agents in the market today. The prime advantage of mAbs as therapeutic and diagnostic agents is their high target specificity, resulting in a low side effect profile. Due to this high therapeutic potential, the process development and production of monoclonal antibodies is being pursued by biopharmaceutical companies globally. Although in the conventional monoclonal antibodies were exclusively produced in hybridoma cells, currently various cell lines are employed for their production of which NS0 and CHO cell lines are the most common, others including murine hybridomas, hamster BHK21 and the human PER.C6 cell line. However, for the choice of the appropriate cell line, certain key criteria have to be considered which include: the ability to provide a close to human glycosylation pattern, the capability to produce high mAb concentrations in the chosen production system, the ability to consistently produce a product of uniform characteristics (stability), and the speed with which a high yielding cell line can be obtained [1,2].

NS0, a murine myeloma cell line, has been adopted by a number of biotechnology companies for the expression of therapeutic antibodies [3-7]. The successful use of NS0 cells in several fusion, transfection, selection and production approaches are among the few properties of this cell line that make it a perfect candidate for the expression of the desired product [8]. However, NS0 cells are known to be susceptible to apoptosis especially under environmental and/or nutritional stress [9].

Lack of Hsp70 expression potential and variability in the membrane cholesterol concentration are among the several factors that are responsible of this particular susceptibility of these cells [10].

A common method for the industrial manufacture of cell culture based recombinant therapeutics is the fed-batch culture. Fed batch cultures have been developed with the objective of achieving maximal increase in the culture viable cell concentrations and prolonging the culture lifetime for increased product concentrations [3,5,11-15] Strategies such as development of the feeding solutions, feeding rate control, maintaining low residual glucose and/or glutamine concentrations, employing dialysis membranes to remove low molecular weight components from the culture, feeding based on Oxygen Uptake Rate (OUR) are employed to achieve the aforementioned objectives. However, the batch operating parameters such as pH, DO, seeding density, temperature are kept constant (or optimized separately) during the fed batch process development. Such approach does not evaluate the interactions between the process parameters (batch and fed-batch) that influence the overall outcome of the process (Figure 1).
Hence, based on the hypothesis that none of the factors affecting the production process can really be independent, fractional factorial design using design expert software was carried out to find the interaction between the batch (Dissolved Oxygen, Temperature and Seeding Density) and fed-batch (post-feed Glucose concentration) process parameters. The usage of design of experiments also facilitates shorter development time, hence more cost effective. This study was carried out to assess the effect of control parameters (post-feed Glucose concentration, temperature, dissolved oxygen, seeding density) on culture response parameters (IVCC, final mAb concentration). The interaction between control parameters and their effects on the production of Humanized-Anti CEA mAb production by NS0 cells in the bioreactor was also evaluated.

Materials and Methods

Cell line and culture medium

The NS0 cell line (Hu2nA33) producing humanized anti- CEA (carcino-embryogenic antigen) was obtained from the Protein Science Department of Inno Biologics Sdn. Bhd. The cells were maintained in cryo vials and stored in liquid nitrogen tanks until usage. The basal medium used to support cell growth was Dulbecco’s Modified Eagle Medium (DMEM, Product # T-043, Biochrom AG).

The supplements added to the basal medium were 2% (v/v) Ultra Low IgG FBS (Product # 16250-078, Gibco), 2 mM L-glutamine (Product # G8540, Sigma Aldrich) and 0.2% (v/v) Pluuronic F-68 (Product # P1300, Sigma Aldrich).

The feeding medium was a 10X concentrated DMEM basal medium (Product # F0455, Biochrom AG) with 4.5 g/L D-glucose and 8 mg/L Phenol red.

Inoculum preparation

Cell culture was initiated by thawing a cryovial followed by subsequent expansion for 1 to 2 weeks using a sequence of spinner flasks (Techne, UK) prior to inoculating into the bioreactor as outlined in Table 1. Throughout the expansion process, cells were maintained in exponential growth phase. Cells were maintained at 37°C in a humid atmosphere of 5% CO2 in an incubator (Galaxy R+, RS Biotech, UK). In order to ensure that all the fed batch runs were inoculated with cells in same physiological state, for each run a new cryopreserved vial was revived. All the cells in the cryopreserved vials were from the same culture flask.

Experimental design

An experimental design was created on DESIGN EXPERT 6.0.8 software (STAT-EASE Inc., US), and half-fractional factorial design (Resolution IV), was used for fed-batch culture incorporating only 12 of the 20 possible combinations (if using full factorial) of 4 selected control parameters at high and low levels. The experiment was designed to be carried out in four blocks (labeled 1, 2, 3 and 4). The blocking was done to ensure that all the experiments were run under homogeneous conditions and shun bias (Table 2). Four centre points was added to the design in order to avoid the risk of missing a curvilinear relationship. Since the experiment design was a Resolution IV design, which meant that no main effect was aliased with any other main effect or with any two-factor interactions, but two-factor interactions are aliased with each other [16]. Thus it was assumed that three factor interactions do not exist or have no significant effect on the output.

### Table 1: General Procedure followed to inoculate the 2L bioreactors.

| Day | Procedure |
|-----|-----------|
| 0   | Cell Revival in 125 mL spinner flask (50 mL working volume) |
| 1   | First Medium Change |
| 2   | Second Medium Change |
| 3   | Subculture in 125 mL spinner flask (50 mL working volume) |
| 5   | Scale upto 250 mL spinner flask (125 mL working volume) |
| 7   | Scale upto 500 mL spinner flask (250 mL working volume) |
| 9   | Scale upto 1000 mL spinner flask (500 mL working volume) |
| 11  | Inoculation into the bioreactor |

### Table 2: Half-fractional factorial design for the validation of process control parameters for fed batch.

| Run No. | Block | A: Post-Feed Glucose concentration (g/L) | B: Seeding Density (x 106 cells /mL) | C: Temperature (°C) | D: Dissolved Oxygen (DO) |
|---------|-------|------------------------------------------|-------------------------------------|---------------------|-------------------------|
| 1       | 1     | 8                                        | 0.3                                 | 35                  | 60                      |
| 2       | 1     | 5                                        | 0.4                                 | 36.5                | 45                      |
| 3       | 1     | 8                                        | 0.5                                 | 35                  | 30                      |
| 4       | 2     | 8                                        | 0.3                                 | 38                  | 30                      |
| 5       | 2     | 2                                        | 0.3                                 | 38                  | 60                      |
| 6       | 2     | 5                                        | 0.4                                 | 36.5                | 45                      |
| 7       | 3     | 2                                        | 0.3                                 | 35                  | 30                      |
| 8       | 3     | 2                                        | 0.5                                 | 35                  | 60                      |
| 9       | 3     | 8                                        | 0.5                                 | 35                  | 60                      |
| 10      | 4     | 5                                        | 0.4                                 | 36.5                | 45                      |
| 11      | 4     | 5                                        | 0.4                                 | 36.5                | 45                      |
| 12      | 4     | 2                                        | 0.5                                 | 38                  | 30                      |
The high and low levels of parameters were as follows: Post-feed Glucose concentration: 2 g/L (11.11 mmol/L) and 8 g/L (44.44 mmol/L) [13] dissolved oxygen tension (DOT) with respect to medium saturation with air (20% and 60%), seeding density (0.3×10^6 cells/mL and 0.5×10^6 cells/mL), Temperature (34°C and 38°C). pH value was set at 7.20 for all the runs.

**Bioreactor equipment**

All the runs were carried out in 2L double wall glass bioreactors (Univessel, B. Braun Biotech, AG). Agitation in the vessel was achieved by standard 65 mm diameter, 45° pitch-blade impeller. The process control parameter set points were maintained using a Digital Control Tower (B. Braun Biotech, AG). The pH was measured using a gas-filled electrode (Mettler-Toledo) and the dissolved oxygen concentration (DO) was measured using a polarographic electrode (Mettler-Toledo). The adjustment of pH was carried out using CO2 gas and 0.5M NaOH. The bioreactor was configured with two mass-flow controllers (Sierra Instruments, Monterey, CA), one for air and one for oxygen. The gas flow rate was held constant while the ratio of air and oxygen was adjusted to maintain the DO set point.

**Fed-batch runs**

Following cell revival, fed-batch experiments were performed in the bioreactor inoculated with an initial cell concentration of 0.3×10^6 cells/mL in 1.5L working volume with 4 mmol/L glutamine and 25 mmol/L glucose. The operating parameters were set according to the experimental design. The experiment duration varied between 5-7 days.

During the initial 2 days of cultivation, bioreactor sampling was performed once daily, taking samples of 5-7 mL. Each day thereafter, sampling was performed twice daily; one before feeding and one within one hour after the feeding. These samples were analyzed for viable cell concentration, percent viability, pH, and biochemical analysis.

**Feeding strategy and feeding medium:** The initial addition of feed medium was performed 2 days after inoculation, once the glucose concentration in the bioreactor was depleted than the intended target post feed glucose concentration values (Figure 3). The feeding strategy was based on maintaining a desired post-feed glucose concentration according to the experimental design. Post-feed target glucose concentrations ranged from 2-8 g/L. The volume of feed medium (V_feed) to be added was calculated using the following equation, as described by Sauer et al. [13], whereas the post feed L-glutamine concentration was maintained at 2 mM [13].

\[
V_{\text{feed}} = \frac{\frac{\text{glc}_{\text{tar}} - \text{glc}}{\text{glc}_{\text{feed}} - \text{glc}_{\text{tar}}}}{V}
\]

where \(\text{glc}_{\text{tar}}\) was the post-feed target glucose concentration, \(\text{glc}\) was the culture’s glucose concentration prior to feeding, \(\text{glc}_{\text{feed}}\) was the feed medium’s glucose concentration, and \(V\) was the culture volume prior to addition.

**Analytical methods**

Viable cell density (VCD) and viability were determined by the trypan blue exclusion method using a haematocytometer. Prior to cell counting, samples were diluted 2 to 10-fold with 0.4% trypan blue solution (GIBCO, USA) depending on the cell density. From the VCD measurement, IVCC was calculated in the same manner as described by Sauer et al. [13].

For biochemical analysis, 5 to 7 ml of the sample were centrifuged for 5 minutes at 3000 rpm to remove cells and further aliquoted and stored at -20°C for metabolites analysis. Glucose, Lactate, and Glutamine concentration were determined enzymatically by the amperometric biosensors of Nova Bioprofile 100 Plus Analyzer (Nova Biomedical Corp., Waltham, MA). Ammonia concentration was determined potentiometrically by the potentiometric sensor of of Nova Bioprofile 100 Plus Analyzer (Nova Biomedical Corp., Waltham, MA). Total humanized anti-CEA IgG titer or monoclonal antibody concentration was determined using the Enzyme Linked Immuno-Sorbent Assay (ELISA) in 96-well micro titer plates as described by Xie and Wang [14]. Human IgG, whole molecule, unconjugated (Pierce, Product # 31154) was used as a standard. First, 100 µL capture antibody solution with a dilution of 1:1000 (anti-human IgG-Fc specific, produced in goat; Sigma, Product # 12136) was placed in a 96-well plate and incubated at 2-8°C overnight. Next, after rinsing with 1×200 µL washing buffer (0.05% Tween-20 in 0.9% NaCl), 200 µL of blocking buffer (0.01% w/v BSA and 0.02% w/v Tween-20 in 1XPBS) was added and incubated for 2 hours at 37°C followed by washing 4X with washing buffer. A 100 µL sample or standard solution was added to each well and incubated for 2 hours at 37°C. After rinsing with 4X200 µL washing buffer, 100 µL labeled secondary antibody, 1.2500 dilution (anti-human gamma chain specific peroxidase conjugate, produced in goat; Sigma, Product # A6029) was added and incubated for another 2 hours at 37°C. The plate was then rinsed with 4X200 µL washing buffer. Then, 100 µL of enzyme substrate working solution (ABTS tablets, Roche, Product # 11 112 442 001, dissolved in ABTS buffer, Roche, Product # 11 112 597 001) was added and incubated for 1 hour in the dark. Absorbance was measured at a wavelength of 405 nm via a kinetic micro plate reader (Bio-Tek Instruments Inc., Highland Park, Winookski). Samples were diluted 1000 to 10,000-fold with blocking buffer prior to assay depending on the antibody concentrations.

**Results and Discussion**

**Batch run**

A batch run was conducted in order to be used as a control and basis for the comparison to fed-batch runs. A maximum cell density of 2.74×10^6 cells/mL was achieved on day 4 of the batch run which resulted in the maximum antibody concentration of 5.21 mg/L on the same day. A biochemical analysis for Glucose and Glutamine revealed that they were depleted from the medium, suggesting that the cells stopped replicating due to nutrient limitation, hence the need for fed-batch process development.

**Fed-batch runs**

Twelve fed-batch runs were conducted according to the experimental design (Table 2), and the summarized data was presented in Table 3 for IVCC, maximum viable cell concentration, cell viability, final mAb concentration, average specific consumption and production for metabolites (glucose, glutamine, lactate, ammonia). Three post-feed target glucose concentrations (2, 5 and 8 g/L) was investigated according to the experimental design (Table 2). These different values resulted in different volumetric feed rates which in turn result in different final working volumes (Table 3) and also affected the cell growth. Four among the 12 fed-batch runs were run at same parameter setting since they were the four centre points in the design. The addition of centre points was done to test for curvature without adding a large number of experimental runs.

The maximum VCD for the 12 runs conducted, ranged from 0.28 to 3.43×10^6 cells/mL with the final mAb titer in the range of 4.96 to...
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Table 3: Data for the fed-batch runs conducted according to the experimental design.

| Run # | Parameter | Value |
|-------|-----------|-------|
| 107.96 | Culture Time (hrs) | 96.24 |
| 22.47 | Final Antibody Concentration (mg/L) | 4.96 |
| 1.28 | Final Working Volume (L) | 1.53 |
| 2.56 | IVCD (x 10^6 cells day/L) | 0.45 |
| 1.4 | Average Specific Antibody Production Rate (mg/10^6 cells per day) | 0.09 |
| 11.1 | Average Specific Glucose Consumption Rate (mg/10^6 cells per day) | 0.68 |
| 3.67 | Average Specific Ammonia Production Rate (mg/10^6 cells per day) | 0.08 |
| 20.23 | Average Specific Lactate Production Rate (mg/10^6 cells per day) | 0.23 |
| 0.78 | Average Specific Ammonia Consumption Rate (mg/L) | 0.98 |
| 35.1 | Final lactate concentration (g/L) | 27.3 |
| 7.03 | Final ammonia concentration (mmol/L) | 3.18 |
| 0.52 | Viable cell concentration at harvest (10^6 cells/mL) | 0.23 |
| 41.08 | Viability at harvest (%) | 38.23 |

59.40 mg/L. Meanwhile, calculated IVCC values ranged from 0.45 to 2.94x10^8 cells day/L. The different values of the response variables are attributed to the fact that the operating parameters were different for the runs. The highest mAb titer of 59.40 mg/L was recorded from Run # 5, indicating an inverse correlation between them.

In comparison to the batch run, Run # 5 improved the mAb yield by 10 fold whereas the maximum cell concentration was increased by 1.25 fold (Figure 2). The improvement can be attributed to the fact that post-feed glucose concentration was maintained at a target value for the fed-batch process development. 

Figure 3 shows the consumption and production profiles for the most important biochemical components in the growth medium namely Glucose, Lactate, Glutamine and Ammonia. The feeding was initiated on day 3 and thus the post feed glucose concentration was maintained at 2 g/L = 11.11 mM (according to the experimental design for Run # 5). The post feed glutamine concentration for all fed-batch runs was maintained at 2 mM. Limiting the availability of glutamine at low concentrations throughout the fed-batch cultivation helps to reduce metabolite accumulation [18,19].

ANOVA approach for determining the interactions

The analysis was performed using the Analysis of Variance (ANOVA) feature of the design expert software. The response variables (IVCC and mAb concentration) of the culture data were modeled with a mathematical function indicating the dependence of these response variables on the various control parameters (Figure 4). The development of such models helps to establish the interactions/main
effects responsible for the process responses. The ANOVA statistic, the F-value or F-ratio, indicates the ratio of the variability in a response caused by changing a control parameter, and the variability caused by random error alone. Larger F-values (>4) indicate the variation explained by the model is greater than would be expected by chance. Values of "Prob>F" less than 0.0500 indicate model terms are significant, whereas values greater than 0.1000 indicate the model terms are not significant. The statistical models relating culture responses to control parameter variation showed highly significant outcomes (F-value>4, p<0.05).

Statistical model for viable cell density (IVCC): The ANOVA F-Value and p-value data for IVCC is shown in Table 4, the F-value and p-values for the model were 17.16 (>4) and 0.0017(<0.01) respectively indicating a good fit for the model.

From Table 4, it is clear that interaction exists between fed-batch operating parameter-post feed glucose concentration (A) and batch operating parameter seeding density (B) and has a significant (F-value=18.98) effect on IVCC. Although the interaction AB could be aliased BC, since the experimental design was Resolution IV fractional factorial design, AB was assumed to be the true interaction since factor

| Source             | F-Value | p-Value | Prob > F |
|--------------------|---------|---------|----------|
| Model              | 17.16   | 0.0017  |          |
| A: Post feed Glucose concentration | 58.14   | 0.0003  |          |
| B: Seeding Density  | 0.16    | 0.7021  |          |
| D: Dissolved Oxygen | 5.66    | 0.0548  |          |
| AB                 | 18.98   | 0.0048  |          |
| AD                 | 2.86    | 0.1418  | 0.9687   |
| Lack of Fit        | 0.08    | 0.9687  |          |
| Curvature          | 0.02    | 0.8970  |          |

Table 4: ANOVA F-Value and p-value for IVCC.
A had a major effect in the model with an F-value of 58.14 and also the factor C didn’t have any effect on IVCC, thus interaction BC was unlikely to be true. At A and B low and D high and the IVCC was at its maximum.

The final equation governing the ANOVA model for IVCC in terms of coded factors was:

\[ IVCC = + 1.44 \cdot 0.64 \cdot A - 0.035 \cdot B + 0.21 \cdot D + 0.38 \cdot A \cdot B - 0.15 \cdot A \cdot D \]  

**eq.1**

**Statistical model for mAb concentration:** The ANOVA F-Value and p-value data for mAb concentration is shown in Table 5. An inverse square root transformation was performed as recommended by the software and the ANOVA model exhibited a significant curvature (F-value being 44.40 (>4) and the p-value of 0.0026 (<0.05), indicating a good fit for the model.

From Table 5, it is clear that interaction exists between fed-batch operating parameter-post feed glucose concentration (A) and batch operating parameter seeding density (B) has a significant effect on the responses mAb concentration. The final equation governing the ANOVA model for mAb in terms of coded factors was:

\[ mAb = + 0.24 - 0.065 \cdot A - 0.017 \cdot B - 0.032 \cdot C + 4.173 \cdot 10^{-3} \cdot D - 0.047 \cdot A \cdot B + 0.016 \cdot B \cdot C \]  

**eq.2**

**Effect of interactions on mAb concentration and IVCC:** The significant interactions AB involved for IVCC and mAb concentrations are shown in Figure 5. The intersection of lines in Figure 5 indicates that factors A and B are involved in the interaction, in case of no interaction, parallel lines would have been observed. The summarized effect of process control variables on these response variables of the fed-batch process is presented in Table 6.

It is evident from Table 4 that for both the response variables (IVCC and mAb concentration), factor A: glucose concentration is the most significant factor and AB interactions are the main driving factors.

The studentised residual versus run number shows random scatter (Figure 6) implying the lack of the effect of run number on culture response variables (IVCC and MAb concentration). The studentised residual is basically a dimensionless statistic used to measure the difference between the actual value of the response observed in the experiment and the predicted value for the response by the experimental model. The studentised residual is used to estimate for the block effect of any of the measured response of a blocked factorial experiment.

The interaction between fed-batch operating parameter-post feed glucose concentration (A) and batch operating parameter seeding density (B) was established and found to be significant for both the culture responses evaluated (IVCC and MAb concentration). The existence of such interactions between fed-batch and batch operating parameters prove the hypothesis that none of the factors affecting the responses of a fed-batch process can be considered independent. Therefore conventional approach to keep batch operating parameters constant while varying only the fed-batch operating parameters needs to be carefully evaluated, since the interactions between the batch and fed-batch parameters are ignored. The knowledge of such interactions during early stages of fed batch process development makes it easier to fully optimize the process. The usage of design expert as a tool for experimental design shortens the early process development and also allows establishing the main factors driving the process response and

**Table 5:** ANOVA F-Value and p-value for mAb concentration.

| Source                        | F-Value | p-Value | Prob > F |
|-------------------------------|---------|---------|----------|
| Model                         | 44.40   | 0.0013  |          |
| A: Post feed Glucose concentration | 139.03  | 0.0003  |          |
| B: Seeding Density            | 9.85    | 0.0349  |          |
| C: Temperature                | 33.09   | 0.0045  |          |
| D: Dissolved Oxygen           | 0.56    | 0.4942  |          |
| AB                            | 70.74   | 0.0011  |          |
| BC                            | 7.79    | 0.0492  |          |
| Lack of Fit                   | 0.08    | 0.7956  |          |
| Curvature                     | 44.40   | 0.0026  |          |

**Table 6:** Summarized of the effects of the investigated control parameters on culture responses.

| Culture Response | A: Glucose Concentration | B: Seeding Density | C: Temperature | D: DO |
|------------------|--------------------------|-------------------|----------------|-------|
| IVCC             | Low                      | Low               | High/Low       | High  |
| mAb Concentration| Low                      | Low               | Low            | High/Low |

**Figure 5:** Interaction between A: Post feed Glucose concentration and B: seeding density for IVCC (a) and mAb concentration (b).
the interactions between them. Resolution IV Experimental design designed using design expert proved an efficient tool for analyzing the fed-batch process, since the number of runs needed to be carried out were 12 (including 4 center points) in comparison to that of a full factorial design (20, including 4 center points). The best among the fed-batch runs conducted increased the final mAb titer by 10 fold in comparison to a batch culture. The maximum VCD value of the developed fed batch process was 1.25 over fold the value for batch.

Conclusion

Fed batch process for the production of anti carcino embryogenic antigen from NS0 hybridoma cells was developed with feeding based on post feed glucose concentration. Design of experiments was used to study simultaneous variation of four different parameters of post feed glucose concentration, seeding density, dissolved oxygen concentration and temperature on cell growth and mAb production. Resolution IV Experimental design designed using design expert proved an efficient tool for analyzing the fed-batch process, since the number of runs needed to be carried out were 12 (including 4 center points) in comparison to that of a full factorial design (20, including 4 center points). The best among the fed-batch runs conducted increased the final mAb titer by 10 fold in comparison to a batch culture. The maximum VCD value of the developed fed batch process was 1.25 over fold the value for batch.

Also the interaction between fed-batch operating parameter-post feed glucose concentration (A) and batch operating parameter seeding density (B) was established and found to be significant for both the culture responses evaluated (IVCC and mAb concentration). The existence of such interactions between fed-batch and batch operating parameters proved the hypothesis that none of the factors affecting the responses of a fed-batch process can be considered independent. Therefore conventional approach to keep batch operating parameters constant while varying only the fed-batch operating parameters needs to be carefully evaluated, since the interactions between the batch and fed-batch parameters are ignored. The knowledge of such interactions during early stages of fed batch process development makes it easier to fully optimize the process.

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References

1. Birch JR, Racher JA (2006) Antibody production. Advanced Drug Delivery Reviews 58: 671-685.
2. Sommerfeld S, Strube J (2005) Challenges in biotechnology production-generic processes and process optimization for monoclonal antibodies. Chemical Engineering and Processing 44: 1123-1137.
3. Bibila TA, Ranucci CS, Glazomitsky K, Buckland BC, Aunins JG (1994) Monoclonal antibody process development using medium concentrates. Biotechnol Prog 10: 87-96.
4. Dempsy J, Ruddock S, Osborne M, Ridley A, Sturt S, et al., (2003) Improved fermentation processes for NS0 cells expressing human antibodies and glutamine synthetase. Biotechnol Prog 19: 175-178.
5. DeZengotita VM, Miller WM, Aunins JG, Zhou W (2000) Phosphate feeding improves high-cell-concentration NS0 myeloma culture performance for monoclonal antibody production. Biotechnol Bioeng 69: 566-576.
6. Whitford W (2003) NS0 serum-free culture and applications. Bio Process Int 1: 36-47.
7. Zhou W, Chen C, Buckland B, Aunins J (1997) Fed-batch culture of recombinant NS0 myeloma cells with high monoclonal antibody production. Biotechnol Bioeng 55: 783-792.
8. Manwaring J, Barnett B, Pence B, Whitford W (2005) NS0 Derivatives: MAB Production in Largescale SFM Formats. Animal Cell Technology Meets Genomics & ESACT Proceedings 2: 581-584.
9. Arden N, Betenbaugh MJ (2004) Life and death in mammalian cell culture: strategies for apoptosis inhibition. Trends Biotechnol 22: 174-180.
10. Lasunskaa EB, Fridlanskaia II, Darieva ZA, da Silva MS, Kanashiro MM, et al., (2003) Transfection of NS0 myeloma fusion partner cells with HSP70 gene results in higher hybridoma yield by improving cellular resistance to apoptosis. Biotechnol Bioeng 81: 496-504.
11. Bibila TA, Robinson DK (1995) In pursuit of the optimal fed-batch process for monoclonal antibody production. Biotechnol Prog 11: 1-13.
12. Ramirez OT, Mutharasar R (1990) Cell cycle- and growth phase dependent variations in size distribution, antibody productivity, and oxygen demand in hybridoma cultures. Biotechnol Bioeng 36: 839-848.
13. Sauer PW, Burky JE, Wesson MC, Sternard HD, Qu L (2000) A High-Yielding,
Generic Fed-Batch Cell Culture Process for Production of Recombinant Antibodies. Biotechnol Bioeng 67: 585-597.

14. Xie L, Wang DIC (1996) High cell density and high monoclonal antibody production through medium design and rational control in a bioreactor. Biotechnol Bioeng 51: 725-729.

15. Zhou W, Rehm J, Europa A, Hu WS (1997) Alteration of mammalian cell metabolism by dynamic nutrient feeding. Cytotechnology 24: 99-108.

16. Meyers RH, Montgomery DC, Anderson-Cook CM (2002) Response Surface Methodology; Process and Product Optimization Using Designed Experiments.3rd Edtn, John Wiley & Sons, New Delhi, India.

17. Hettwer DJ, Escobar E, Fieschko J (1991) Development of a serum free suspension process for recombinant CHO cells. Presented at the American Institute of Chemical Engineers Annual Meeting, Los Angeles CA, USA.

18. Xie L, Daniel ICV (1997) Integrated approaches to the design of media and feeding strategies for fed-batch cultures of animal cells. Trends Biotechnol 15: 109-113.

19. Xie LZ, Wang DIC (1994) Fed-Batch Cultivation of Animal Cells Using Different Medium Design Concepts and Feeding Strategies. Biotechnol Bioeng 43: 1175-1189.