Statistically Designed Optimization for Monoclonal Antibody Production in CHO Fed-Batch Culture

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Abstract

Statistically designed optimization has found use in a variety of industrial applications where a process outcome is influenced by several variables. It can maximize process yields while reducing the number of experiments required to adequately sample process space. We report the use of response surface methodology for rapid development of a fed batch process for a CHO-derived fully human monoclonal IgG_1 antibody. Response surface methodology was used to optimize the process parameters and maximize titer.

Cells were first grown to high density under standard conditions and then shifted to production conditions several days post seeding. Six process-shift parameters were examined: temperature, pH, dissolved oxygen (DO) level, and the time of shift (days post seeding) for each parameter. Using SAS JMP 5.01 (SAS JMP, Cary, NC) 47 experiments were designed to sample process space and create response surface model for each output variable: integrated viable cell density (IVCC), volumetric product concentration (P), specific productivity (qP) and several metabolic rates. Experimentation was executed in 2L bioreactors (DASGIP AG, Jülich, Germany) in six experimental blocks and completed over a six week period. The optimal shift-parameters were central-point (midpoint) conditions over the range studied.

Introduction

Overview

- Process development of a fed batch process requires development and optimization process parameters and investigation of process control strategies to achieve high volumetric titers.
- The process is usually divided into a growth phase in which cells quickly grow to high density and a production phase in which growth is limited and production increases.
- Here, we have used surface response statistical design to aid in determining appropriate growth conditions and process shift parameters to induce production phase.

Purpose of Study

- Previously, an analogous process was developed for the IgG_2 version of CR011, a fully human monoclonal antibody against melanoma antigen GPNMB.
- Here, we use process parameters suggested by the IgG_2 study in conjunction with statistical design to optimize the IgG_1 process in 6 weeks.
- Process parameters (temperature, pH and dissolved oxygen concentration) during growth phase, process shift values (temperature, pH and dissolved oxygen) to induce production phase as well as time of shift were simultaneously optimized in a 2L bioreactor format.

Background

- Response surface designs are useful for modeling a curved surface to continuous factors.
- A response surface model can determine a minimum or maximum response inside the factor region, if one exists.
- Response surface models allows simultaneous optimization of several parameters by predicting surface shape using a small number of experimentally determined responses over the parameter range. Alternative, full factorial designs are prohibitively costly and time consuming.

Materials and Methods

- **Cell Culture:** A recombinant Chinese Hamster Ovary cell line expressing a fully-human IgG_1 antibody against glycoprotein NMB (GPNMB) was cultured in 2 L DAS-GIP. The temperature was maintained at 37°C during growth phase and then shifted during production phase. The pH was automatically controlled at 7.0 by adding 1M Na_2CO_3 or sparging CO_2 gas. The dissolved oxygen (DO) was controlled at 40% by changing the inlet oxygen proportion in the sparge gas mixture. pH and DO were also shifted to production conditions as determined by statistical design. Agitation was maintained at 100 rpm with a marine impeller. Glucose and glutamine were maintained throughout the culture with manual feeding. Feed media was added into the bioreactor at day 4, 7 and 10 post-seeding.

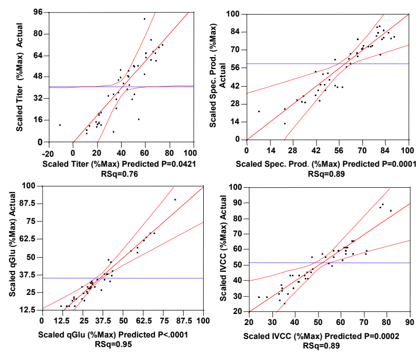
- **Statistical Design:** The statistics software SAS JMP was used to generate a central composite response surface design using three process parameters (temperature, pH and dissolved oxygen) and a time of shift parameter for each process parameter. As a result, 47 experiments were designed (below) with over a parameter range (-1, minimum to +1, maximum).

Design		Temp Shift	Time of Temp Shift	pH Shift	Time of pH Shift	DO Shift	Time of DO Shift
1	A00000	1	0	0	0	0	0
2	***0***	***0***	***0***	***0***	***0***	***0***	***0***
3	+-+--+	1	-1	1	-1	-1	1
4	a00000	-1	0	0	0	0	0
5	++++-+	1	1	1	1	-1	1
6	00000a	0	0	0	0	0	-1
7	-----+	-1	-1	-1	-1	-1	1
8	+-----	1	-1	-1	-1	-1	-1
9	+--++-	1	-1	-1	1	1	-1
10	+-+-+-	1	-1	1	-1	1	-1
11	--+-++	-1	-1	1	-1	1	1
12	-+-+-+	-1	1	-1	1	-1	1
13	***0***	***0***	***0***	***0***	***0***	***0***	***0***
14	0000a0	0	0	0	0	-1	0
15	++--+-	1	1	-1	-1	1	-1
16	-+----	-1	1	-1	-1	-1	-1
17	--+---	-1	-1	1	-1	-1	-1
18	0A0000	0	1	0	0	0	0
19	-+++--	-1	1	1	1	-1	-1
20	+++-++	1	1	1	-1	1	1
21	+-++++	1	-1	1	1	1	1
22	++---+	1	1	-1	-1	-1	1
23	***0***	***0***	***0***	***0***	***0***	***0***	***0***
24	+++---	1	1	1	-1	-1	-1
25	00000A	0	0	0	0	0	1
26	----+-	-1	-1	-1	-1	1	-1
27	00A000	0	0	1	0	0	0
28	00a000	0	0	-1	0	0	0
29	--+++-	-1	-1	1	1	1	-1
30	0a0000	0	-1	0	0	0	0
31	++-+++	1	1	-1	1	1	1
32	-++-+-	-1	1	1	-1	1	-1
33	+---++	1	-1	-1	-1	1	1
34	++-+--	1	1	-1	1	-1	-1
35	000A00	0	0	0	1	0	0
36	0000A0	0	0	0	0	1	0
37	-+++++	-1	1	1	1	1	1
38	000a00	0	0	0	-1	0	0
39	+-++--	1	-1	1	1	-1	-1
40	---+--	-1	-1	-1	1	-1	-1
41	---+++	-1	-1	-1	1	1	1
42	+--+-+	1	-1	-1	1	-1	1
43	+++++-	1	1	1	1	1	-1
44	-+-++-	-1	1	-1	1	1	-1
45	--++-+	-1	-1	1	1	-1	1
46	-++--+	-1	1	1	-1	-1	1
47	-+--++	-1	1	-1	-1	1	1

- The experiments were conducted in two sets of eight 2 L bioreactors in parallel to provide an experimentally determined sampling of the response surface. The center points with all '0' conditions served as a control for each run. Samples were taken and analyzed to determine cumulative integrated viable cell count (IVCC), product concentration (P), specific productivity (qP), and metabolic rates (qGlu, qLac, qGln, and qNH_4) on the final day of fed-batch culture. The variations among data were normalized according to central-point data in each run and were entered in SAS JMP statistics software to fit into response surface model. The response surface coefficients were then estimated by the software for each output variable: IVCC, product concentration, and metabolic rates (28 coefficients for each output) to fit the response surface model:

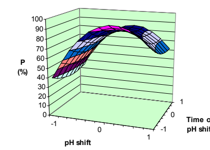
$$\text{Response surface} = \beta + \sum \alpha_i X_i + \sum \alpha_{ij} X_i X_j \quad \text{where } i \le j$$

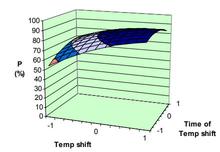
Results

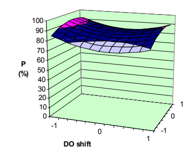


- Reasonable R^2 values and low P values indicate that the response surface model accurately fits the experimental data. The regression line (solid red) and the 95% confidence curves (dashed red) cross the sample mean (dashed blue), which show that the model fits a significant proportion of the data.

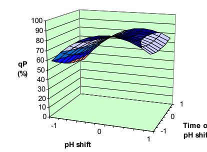
Volumetric productivity (P, percent of maximum) response surface

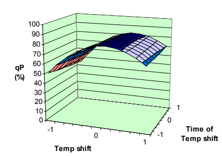


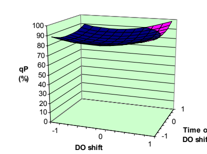




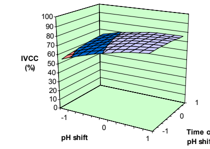
Specific productivity (qP, percent of maximum) response surface

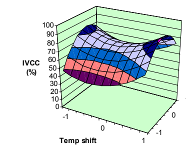


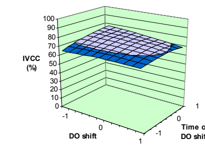




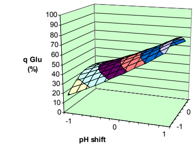
The cumulative integrated viable cell density (IVCC, percent of maximum) response surface

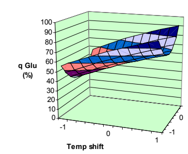


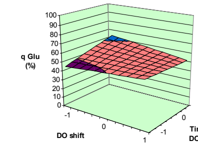




Glucose consumption (qGlu, percent of maximum) response surface







Discussion

- The product concentration shows that the central points were optimal for productivity where pH, temperature, and DO were shifted to midpoint conditions (0,0,0) at time point 0.
- Specific productivity (qP) was also maximized at center point conditions shifted at time point 0. DO shift had a small effect on this parameter.
- The response surfaces of the cumulative integrated viable cell density (IVCC) indicated that temperature and time of temperature shift had the greatest effect on this parameter. Shifted pH, and DO and shifting time for pH and DO had only a small effect on IVCC. The highest IVCC was achieved when temperature was shifted at time point 0.
- Response surface fits for metabolite production and nutrient consumption are also informative. For example, cumulative glucose consumption response surfaces show that glucose consumption increased with temperature and pH. DO had a less significant effect on this parameter.

Summary

- A fed batch cell culture process was optimized in 6 weeks using sixteen 2L bioreactors.
- Response surface methodology allows characterization of growth, productivity and metabolic response to changes in process parameters (temperature, pH, and dissolved oxygen) without conducting a full-factorial experiment.