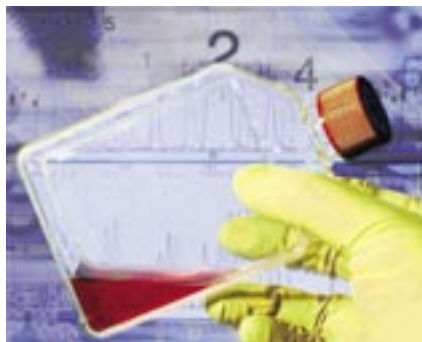


Determining the Effect of Raw Materials on Manufacturing-Scale Cell-Culture Performance

An Application of Mechanistic Mathematical Models

Siddhartha Jain, Bernhard M. Schilling, and Abhinav A. Shukla

Large-scale cell culture production processes routinely involve multicomponent cell culture media formulations including both chemically defined raw materials and complex raw materials such as hydrolysates (1). Even minor variations in the compositions of either can lead to variability in protein productivity or product quality. That often persists despite the use of a raw material lot-blending strategy at large scales to “average out” raw material trends. And a raw material lot-blending strategy can make it more difficult to identify which single media component is responsible for a variation. Analysis of cell culture processes to identify their critical raw materials is further complicated by use of multiple raw materials that may interact with each other as well as multiple formulations that are mixed together to create a final basal or feed medium. Identification of a raw material component causing variability in cell



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culture performance is a critical first step toward establishing better control over cell culture processes at manufacturing-scale.

Statistical tools such as analysis of variance (ANOVA) and chemometrics are commonly used in analyzing cell culture performance. Chemometric tools such as principal component analysis (PCA), partial least squares (PLS), and soft independent modeling of class analogy (SIMCA) can be used to analyze a large number of variables together, so they are effective in situations that involve multistage operations, multiple raw materials, and large datasets (2–4). These tools have been applied for process control and variability analysis in bacterial and mammalian cell culture processes, but they lack the mechanistic details associated with complex biological systems (5). Here, we describe a multivariate mathematical tool that fits cell culture performance parameters to

mathematical models that are mechanistic in nature and can thus be used to identify the most critical raw material in a cell culture process.

THEORY

We used three mechanistic mathematical models for our mathematical analysis: cooperative, additive, and substitutive. These represent the generalized forms of interactions that occur in biological systems (6, 7).

The cooperative model assumes that raw materials interact with each other. Equation 1 describes their effects on protein production according to the cooperative model. The additive model assumes that raw materials participate independently and often in parallel with each other. Equation 2 describes the relationship between titers and contributions from the raw materials according to the additive model. The substitutive mechanism assumes that raw materials participate in the same pathway, so their effects can be added together as shown in Equation 3.

Algorithm of the Mathematical

Analysis: Figure 1 shows the algorithm we developed to correlate titers with raw material use. It comprises three steps: parameter estimation, calculation of individual raw material contributions, and determination of correlation coefficients.

Using the Solver function in Microsoft Excel, parameters A_p , B_p , and C_i were estimated for the least-squared

PRODUCT FOCUS: PRODUCTS OF MAMMALIAN CELL CULTURE

PROCESS FOCUS: PRODUCTION

WHO SHOULD READ: QA/QC AND PROCESS DEVELOPMENT

KEYWORDS: MEDIA OPTIMIZATION, CELL CULTURE PROCESS OPTIMIZATION, RAW MATERIALS ANALYSIS, LARGE-SCALE

LEVEL: INTERMEDIATE

Equations 1-3: T_{max} = maximum titer achievable during protein production; K = half-maximum contribution from raw materials; A_i, B_i, C_i, \dots = contribution from individual lots of raw materials A, B, C, ...

$$Titer = T_{max} \left(\frac{A_i}{K_A + A_i} \right) \left(\frac{B_i}{K_B + B_i} \right) \left(\frac{C_i}{K_C + C_i} \right) \dots$$

Equation 1

$$Titer = T_{max} \left[\left(\frac{A_i}{K_A + A_i} \right) + \left(\frac{B_i}{K_B + B_i} \right) + \left(\frac{C_i}{K_C + C_i} \right) + \dots \right]$$

Equation 2

$$Titer = T_{max} \left(\frac{(A_i + B_i + C_i + \dots)}{K + (A_i + B_i + C_i + \dots)} \right)$$

Equation 3

error between simulated and actual titers in individual mathematical models. Those parameters were inserted into their corresponding models, one raw material at a time (e.g., only A_i parameters inserted for contribution from raw material A, and so on), to determine relative contributions from each. We determined correlation coefficients between the individual raw material contributions and titers at manufacturing scale for each of our three mathematical models. Then we tested those correlation coefficients against the hypothesis of no correlation using t -test at a 0.05 significance level.

For each mathematical model, we compared significant correlation coefficients with each other and determined p -values (8). The critical raw material was identified in each case based on associated statistical significance ($p < 0.05$).

For results reported here, we applied the cooperative model to determine the effect of raw materials on cell culture performance at commercial manufacturing site 1. Similar analysis was conducted at commercial manufacturing site 2, which involves raw material blending (data not shown). The mathematical tool was successfully applied to situations involving such lot blending. Parameters A_i, B_i , and C_i were weighted for estimation according to raw material use. Results obtained from additive and substitutive models at both sites were identical to those from the cooperative model.

RESULTS AND DISCUSSION

During production of a recombinant protein at two commercial manufacturing sites, an upward drift in protein titers was simultaneously observed (Figure 2). It happened simultaneously at two independent commercial manufacturing sites with no process or equipment modifications made, so raw materials were suspected to be the cause. Analysis of cell culture media at both sites revealed the use of three multicomponent raw material formulations (A, B, and C). Because of their complexity, they were

identified as likely root causes for the protein titer variations. Below we discuss methods for identifying which formulation is responsible. A raw material lot-blending strategy was used at site 2, whereas single lots of each raw material were used during each manufacturing run at site 1. The blending done at site 2 made it difficult to conduct a simple statistical analysis, and ANOVA applied at site 1 gave inconclusive results (data not shown).

Mathematical Analysis of Cell Culture Performance: Protein titers at commercial manufacturing site 1 were fitted to the cooperative mathematical model, and

parameters A_i, B_i , and C_i were obtained based on least-squared error using the Solver function in Microsoft Excel. For parameter estimation, we used $K_A = K_B = K_C = 0.1$, and T_{max} was chosen as a value close to the historical maximum titer. Analytical results remained unchanged when the K and T_{max} values were changed. Initial parameter estimates were taken as 0.01; the results did not change for estimates between 0.001 and 0.1. Figure 3 shows protein titers and simulated titers obtained from the best fit for the cooperative model.

We obtained the contributions of each raw material by inserting the

Figure 1: Algorithm used for mathematical analysis of cell culture performance

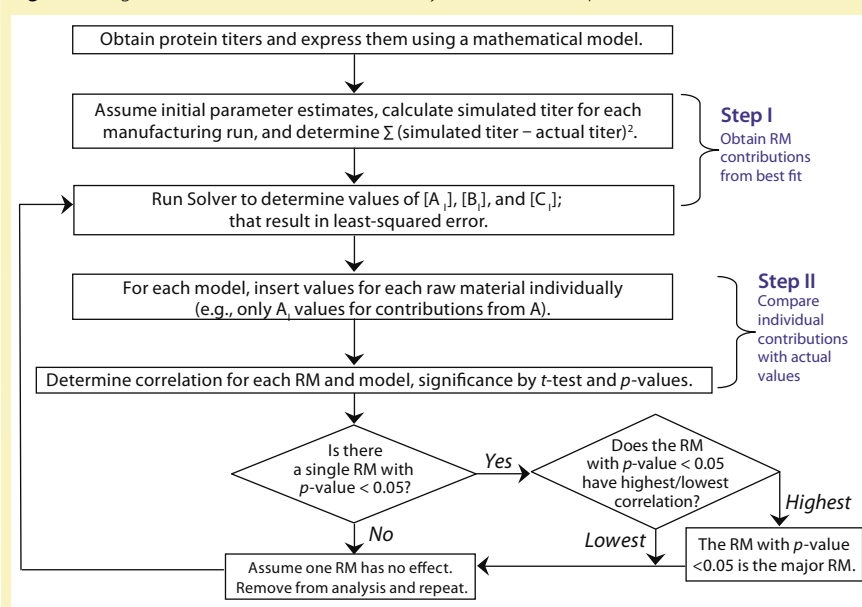


Figure 2: Protein titers observed at commercial manufacturing sites 1 and 2

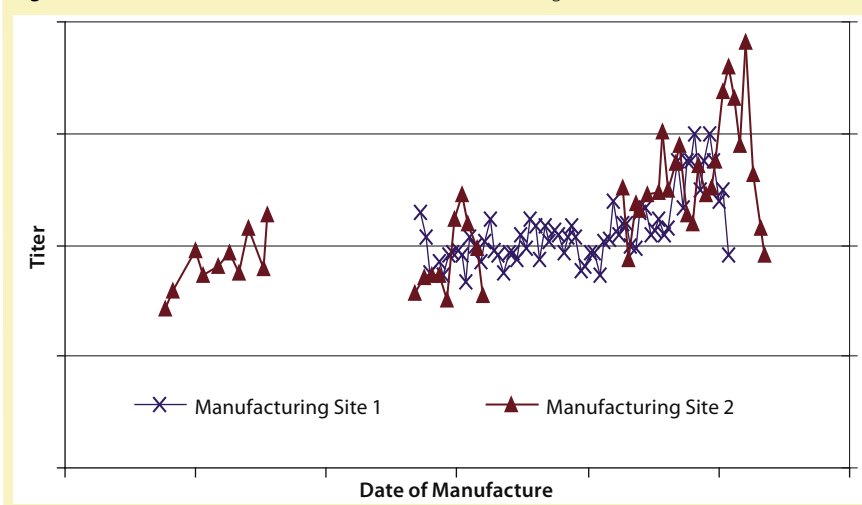


Figure 3: Comparing protein titers and simulated titers for the cooperative model at commercial manufacturing site 1

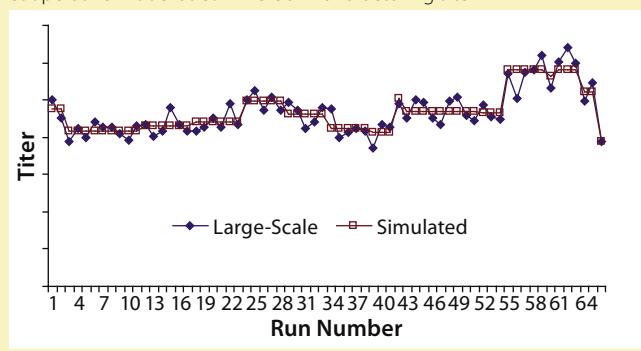


Figure 4: Comparing protein titers and contributions from each raw material at commercial manufacturing site 1

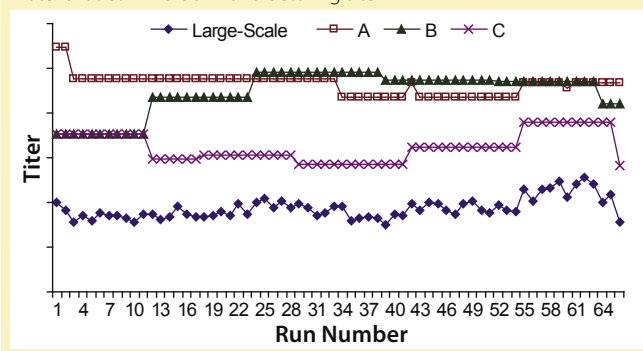


Figure 5: Correlation coefficients comparing protein titers and raw material contributions for all three raw materials at commercial manufacturing site 1

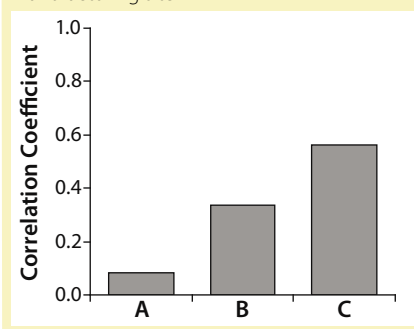
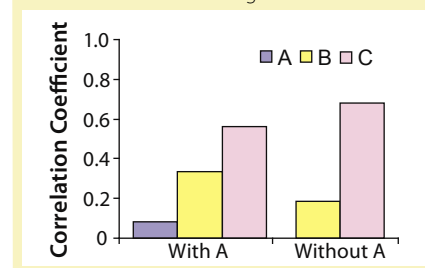


Figure 6: Correlation coefficients for raw materials A (blue), B (yellow), and C (pink) with and without analyzing raw material A at commercial manufacturing site 1



parameters estimated during the least-squared error fit into Equation 1 for each raw material individually. Figure 4 overlays raw material contributions and titers at manufacturing scale. And Figure 5 shows correlation coefficients determined between raw material contributions for each raw material and titers at manufacturing-scale.

Correlation coefficients for the three raw materials resulted in t -values of 0.7, 2.9, and 5.5 respectively. Because the t -value of raw material A is less than 2.0 (p -value of 0.05, degree of freedom 64), and the t -values for raw materials B and

C are greater than 2.0, only raw materials B and C were considered to have significant correlations to titers. Raw material C had the highest correlation coefficient (Figure 5) but was not significantly higher statistically when compared with raw material B (p -value = 0.2).

Because raw material A had no significant correlation with titers based on the t -test, we repeated our analysis without it. Figure 6 shows correlation coefficients for this second analysis. Raw materials B and C had t -values of 1.5 and 7.4, respectively. So we determined that only raw material C is positively correlated to titers at commercial manufacturing scale (t -value > 2.0). Thus, raw material C has more effect on protein productivity than materials A and B. Analysis conducted at commercial manufacturing site 2 also showed C as the most critical raw material in the cell culture process.

Applicability of this Multivariate

Analysis for Process Control and Optimization: The ability of the mathematical tool to correlate performance parameters to raw material use suggests a wide range of applications in the analysis of mammalian cell culture. It may be applied to analyze the effect of raw materials on a number of performance parameters such as product quality, cell growth profiles, and metabolite formation. Identification of critical raw materials can lead to process improvement efforts for more consistent cell culture performance. This mathematical tool also can provide better insight into how raw materials affect performance at commercial scale and help us understand how raw materials can interact.

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Siddhartha Jain is an upstream engineer, **Bernhard M. Schilling** is upstream group leader, and corresponding author **Abhinav A. Shukla** is associate director of manufacturing sciences at Bristol-Myers Squibb Company, PO Box 4755, East Syracuse, NY 13221; 1-315-431-7926; abhinav.shukla@bms.com.