Optimizing Process Parameters Simultaneously

Design of Experiments in Bioprocess Development

Ulrike Becken

In bioprocessing, a complex interplay of different parameters determines the progression of the process and the formation of the desired endproduct. To develop efficient and robust processes, bioprocess engineers need to understand these interdependencies. They can achieve it using Design of Experiments, a method that can uncover complex relationships while saving resources.

Bioprocesses are like an equation: On the left side of the equation there are the process parameters —temperature, medium pH, concentrations of nutrients and byproducts, and others—that interact in a particular way. On the right side there is the process result, which is described by the amount of biomass, product concentration, product quality, the appearance of impurities, and so on (Fig. 1).

An understanding of this equation allows the bioprocess engineer to optimize the process without having to rely on trial and error. Three questions are particularly important: Which process parameters influence the result? What are the optimal setpoints? How do the process parameters play together?

There are several experimental strategies to answer these questions. Traditionally, one factor is changed per experiment (One Factor a Time, OFAT). For example, different temperature setpoints are tested while keeping all other parameters constant (Fig. 2A). This approach has major disadvantages, however. First, many experiments are needed to find optimal setpoints. Furthermore, the results do not indicate interactions between the process

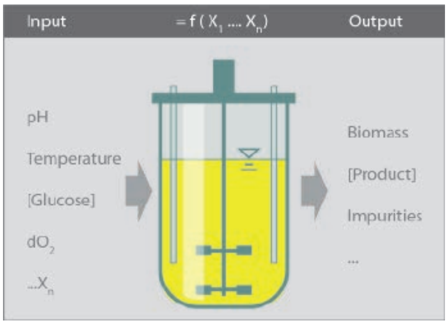

Fig. 1: Many parameters influence the process result. Understanding the interdependencies is fundamental to optimizing bioprocesses.

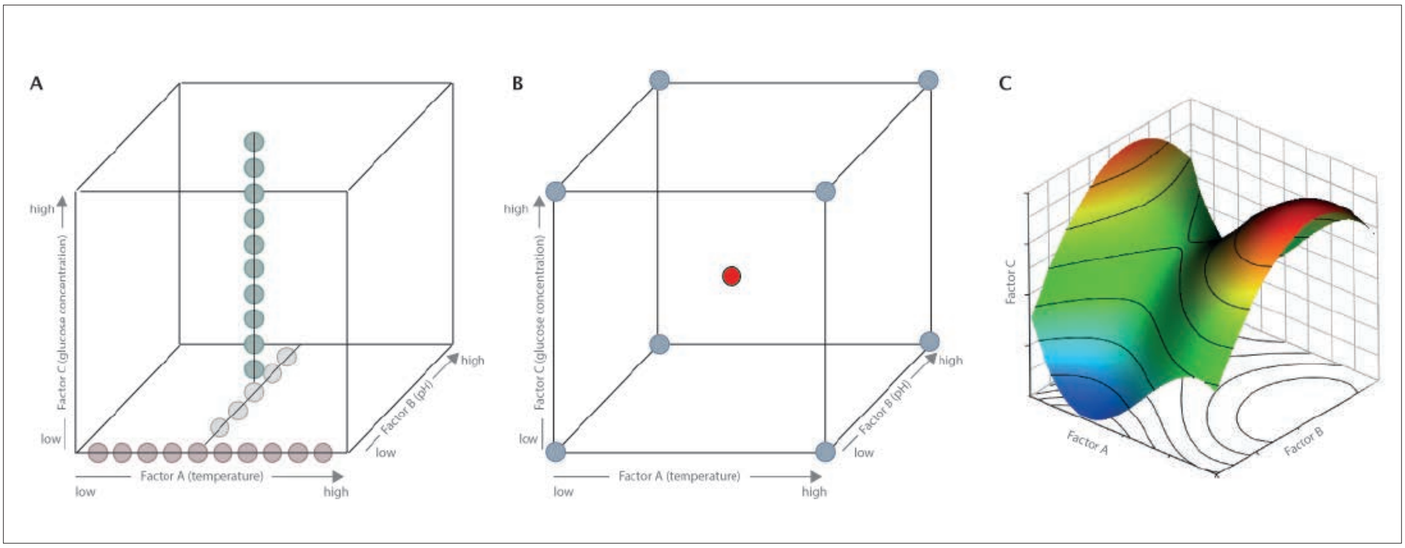

Fig. 2: Experimental design and analysis A. OFAT approach. Each circle represents an experiment. In each experiment, one factor is varied while the others are kept constant. One can only optimize only one parameter at a time. B. DoE study. Each circle represents an experiment. In each experiment, three factors (pH, temperature, glucose concentration) are varied. Compared to the OFAT approach, a larger range of parameters is covered with fewer experiments. By describing the relationships with the help of mathematical models, knowledge is gained within the cube. C. The Surface Response Plot is a way to visualize the results of a DoE study. It illustrates which combination of setpoints provides better (red) or worse (blue) results (Figure: CAMO Software AS).

parameters. An example: Suppose that in an OFAT approach temperature setpoints between 30° C and 37° C are tested; the medium always has a pH of 7.0 and contains 2 g/L of glucose. Let's assume that a temperature setpoint of 35° C results in the highest product concentration. The problem is that this is probably not the true optimum. The result might be better if several factors are changed. For example, at 32° C the product concentration may be even higher if the medium pH is set at 6.8 and the glucose concentration to 4 g/L at the same time. Classic OFAT approaches do not provide information about such interdependencies.

Determine critical parameters and recognize interdependencies

A Design of Experiments approach bypasses such problems. In each experiment several factors are changed simultaneously. That may seem counterintuitive at first, but if done in the right way it allows a wider range of parameters to be covered, unravels interdependencies, and gives hints for meaningful follow-up experiments. A great advantage of a DoE approach is that only the experiments that are really needed are done, and so resources are saved.

Figure 2B schematically illustrates a DoE design to study the influence of temperature, pH, and glucose concentration on the process result. For each parameter, two setpoints are tested, a low one and a high one. In the first experiment, the low setpoints for pH, temperature and glucose concentration are chosen. In the next experiment pH and temperature are low, but the glucose concentration is high. According to this scheme all possible combinations of high and low setpoints are tested - their number corresponds to 2^k, with k being the the number of parameters to be tested. In addition, several experiments are often performed at the Center Point (Fig. 2B, red) to estimate the power of the design and to determine standard deviations. This is called a Full Factorial Design. Depending on the question, it may make sense to modify or extend this design, for example, by testing additional setpoints.

In our example, not just one but three process parameters are varied in each experiment.

How do scientists know what setpoint range is suitable? Experience and literature research give a first idea. A great strength of DoE studies is that they allow conclusions to be drawn about meaningful adjustments. If, for example, the combination of pH high/temperature high delivers the best process results, the setpoint range should be shifted upwards in a follow-up DoE study.

The data obtained from a DoE study is statistically analyzed. In this way, the scientist can draw conclusions as to whether some process parameters interact and which parameters have the greatest influence on the result. Furthermore, a setpoint range can be defined, within which the process result is satisfactory. There are various possibilities to visualize the results. A surface response plot, for example, provides a kind of map of the process (Fig. 2C).

Specialized software products simplify tasks

DoE design and statistical analysis are usually done using specialized software. Products that work together seamlessly are especially user-friendly. Such packages allow easy transfer of data between the bioprocess control software and the software for DoE design and analysis. Parallel bioreactor systems are also very useful tools, allowing many experiments to be performed at the same time, thus speeding up the process.

DoE studies facilitate the development of a comprehensive process understanding with minimal experimental effort. They help to identify important process parameters, optimize processes, and predict how changing a process parameter will influence the the result.

Contact
Dr. Ulrike Becken
Eppendorf AG Bioprocess Center
Jülich, Germany
becken.u@eppendorf.com

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