Bioreactor Monitoring, Modeling, and Simulation

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Mathematical model–based simulations of actual bioreactor runs suggest how process variables such as substrate and product concentrations change and how nutrient feeding should be "tuned" with respect to time, pattern, concentration, and composition to elicit a desired response. Insights gained from modeling can guide us in the adjustment of a process, reducing the number of characterization rounds required. Furthermore, comparing actual experimental results with model predictions helps improve the models themselves. It is important to note that outputs can vary in unpredictable ways if processes are simulated outside boundaries set by the models that describe them, especially if the true operating ranges of actual processes are inadequately captured (**58**).

Figure 3 lists bioreactor operational modes used in bioproduction, which are prerequisite to appreciating the differences in modeling approaches. The modeling of cellular productivity in bioreactors presents a formidable challenge because of many inherent high-degree nonlinearities (especially in batch and fed-batch culture modes), which are ultimately related to the complexities of living cells and the dynamics of in vitro culture. In response to changes in their culture environment, and driven by their genetic information, living cells alter the rate of their biochemical reactions (or the nature of those reactions) by, e.g., inducing new enzymes while repressing existing ones in an effort to find a renewed cellular homeostasis. The overall effect of the underlying mechanisms of cellular regulation dictates a nonlinear behavior in cell culture processes.

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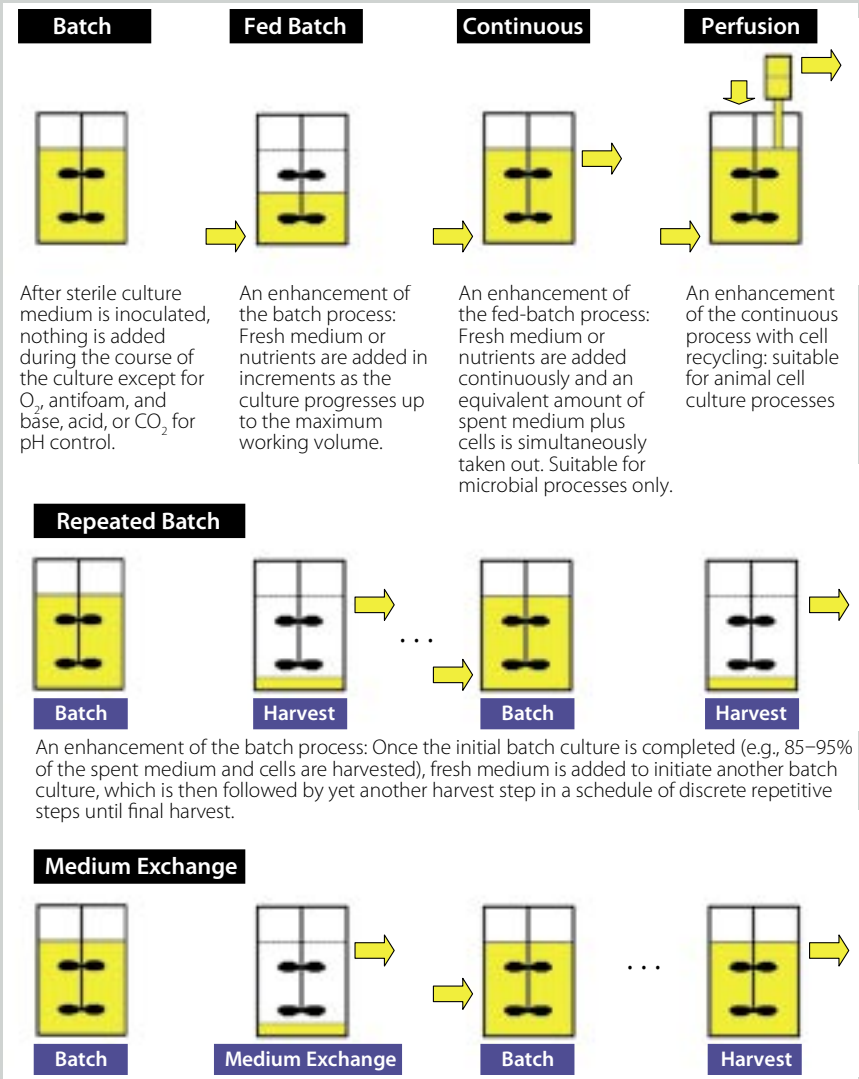
The prevalence of fed-batch or repeated fed-batch operation in most commercial cell culture manufacturing processes means that most producers are operating not only in an absence of the true steady state, but also under conditions in which many individual processes follow multiple, divergent trajectories through the operational cycle. This adds to the complexity of modeling such operations. Early modeling experiences from industrial microbial culture processes (**22**) are of rather limited and merely conceptual modeling value for our fed-batch processes. They are predominantly operated in the "steady state" continuous mode (**23–30**) and rely on the optimization of steady-state culture conditions. That comes from the difference in control objectives between the two types of culture. In continuous mode, the objective is to maximize the amount of desired product per unit time, whereas in batch or fed-batch modes the goal is to maximize product at the end of each batch, leading to control challenges of a different nature.

Note that another culture mode is sometimes used in industrial settings: perfusion cell culture. Although continuous by design, perfusion requires cell retention devices (**31–33**) that present yet another category of challenges for dynamic modeling (**34**). Judging from the limited publication of results so far, this technique is still in its infancy (**35**).

Some unstructured models have been developed for fed-batch microbial (**36–39**) and mammalian cell culture (**40–43**) processes that model cell growth, substrate consumption, and product formation. Some describe the use of (artificial) neural networks (NN) and fuzzy logic (FL). Those latter so-called "expert" systems (**44–56**) are introducing intriguing possibilities.

Furthermore, reliable sensor technologies are seldom found that can provide real-time assessment of many intra- and extracellular activities. Those currently available tend to suffer from high complexity, insufficient accuracy, risk of contamination, or insufficient robustness altogether, which makes inherently dynamic process states very difficult to characterize.

Figure 3: Classification of bioreactor operational modes — batch culture is the only closed culture mode. Fed-batch, continuous, and perfusion culture are semicontinuous or continuous modes of operation as compared with repeated batch and medium exchange, which are discrete culture modes of operation.



Despite all those difficulties, process modeling has gained enormous popularity among bioprocessors because it so facilitates process optimization. That holds the promise of affecting quite an array of production characteristics, including increasing product quality and reducing manufacturing cost (57), risk, and time. In meeting such objectives, valuable process models must capture the dynamic, multivariate, and nonlinear information that enables prediction of process trajectories and helps us deal effectively with the consequences of changing process load conditions. Readers unfamiliar with the nonlinear modeling classification and its techniques can refer to the "Foundations in Bioreactor Process Modeling" box.

Process modeling is both a science and an art because a good dose of creativity is required to make assumptions for a computationally simple yet predictive model. Modeling inherently involves a compromise between accuracy (complexity) and the cost and effort involved in developing a model. Even after having determined the boundaries and validating a model, preferably with

Foundations in Bioreactor Process Modeling

Figure 4 (next page) depicts a nonexhaustive classification of model forms useful in bioreactor modeling and simulation.

Qualitative Models and Fuzzy Logic: A qualitative model often can be formulated even when the course of a culture process is not amenable to mathematical modeling. For example, discontinuities (e.g., induction steps that require a culture to be operated at abruptly changing operating regions or discrete time and volume changes) occur during medium exchange in repeated-batch culture. The simplest form is the "rule-based" model that makes use of "IF–AND–THEN–ELSE" language to describe process behavior. Such rules are often elicited from human experts (skilled operators). For example, rather than attempting to model feed requirements mathematically, terms are used such as "IF glucose level is too low AND cellular oxygen uptake rate is high THEN add glucose ELSE do not add glucose."

"Fuzzy logic" (FL) is intended to rectify disadvantages in purely rule-based models by invoking some form of algebra to enhance accuracy. In particular, popular FL algorithms combine algebra with linguistics to facilitate descriptions of complex systems and cope effectively with process uncertainty. Fuzzy reasoning incorporates real-world system knowledge into a model and uses sets of "partial membership," instead of traditional data sets. Those are then qualified as true or false depending on whether each element is or is not included in a particular data set.

Mechanistic Models: Among the most commonly used are mechanistic models derived from fundamental physics, chemistry, and biology governing a process. Equations describing process conditions are developed from two basic sources: metabolite and recombinant product level values from actual bioreactor experiments (heuristic); or the more theoretical mass/energy conservation balances and kinetics of metabolic reactions (deterministic). A set of nonlinear ordinary differential equations (ODE) and/or partial differential equations (PDE) with related algebraic equations are compiled to produce mathematical models that simulate real systems.

continued

Foundations in Bioreactor Process Modeling, continued

ODEs refer to lumped parameters and are used to describe behavior in one dimension (normally time), whereas PDEs refer to distributed parameters and account for spatial differences (e.g., substrate gradients in large bioreactors). A distributed parameter model can be considered as unstructured and segregated because, although it accounts for spatial differences within a bioreactor, it is still governed by the same unstructured model equations that describe what an entire subpopulation does in that particular area. Although distributed parameter models are more complex and more difficult to develop and solve, their significance is amplified in large bioreactors where, e.g., mixing kinetics and times may become critical to successful bioreactor operation.

"Black Box" Models and Neural Networks (Expert Systems): The terms *black box* and *empirical* models simply describe the functional relationships between system inputs and system outputs. The algorithm parameters involved do not necessarily have any physical meaning in terms of equivalence to actual process variables, which present an obvious limitation. However, they can often accurately model process trajectories.

Neural networks (NN) are particularly suited to modeling complex nonlinear processes. Whereas conventional computerized approaches solve control problems based on algorithms using a cognitive computational approach (the algorithm to the problem must be understood a priori, or the computer cannot solve it), neural networks take a completely different approach. They are composed of a large number of highly interconnected processing units working in parallel, a concept originally inspired by the way the human brain processes information (Figure 5). Here, the number of component input and output "nodes" used is determined by the nature of a process modeling problem being tackled along with the input data representation and the form of the required output.

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Figure 4: Classification of nonlinear model forms used in bioreactor modeling

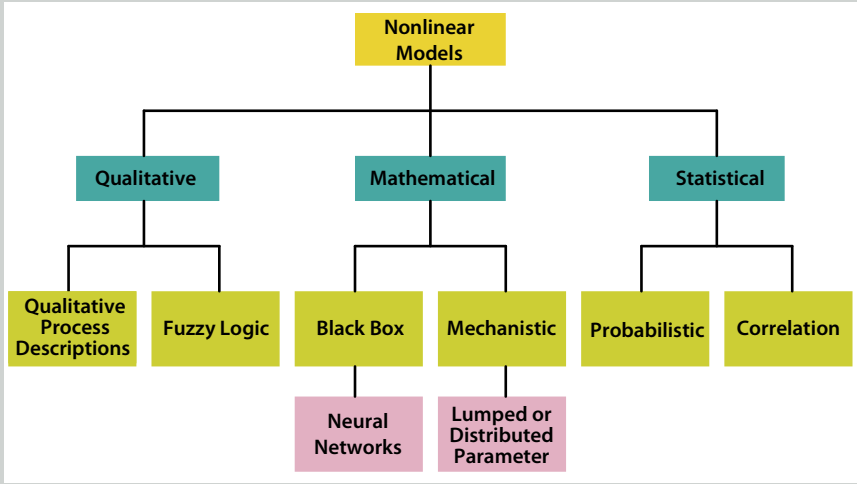
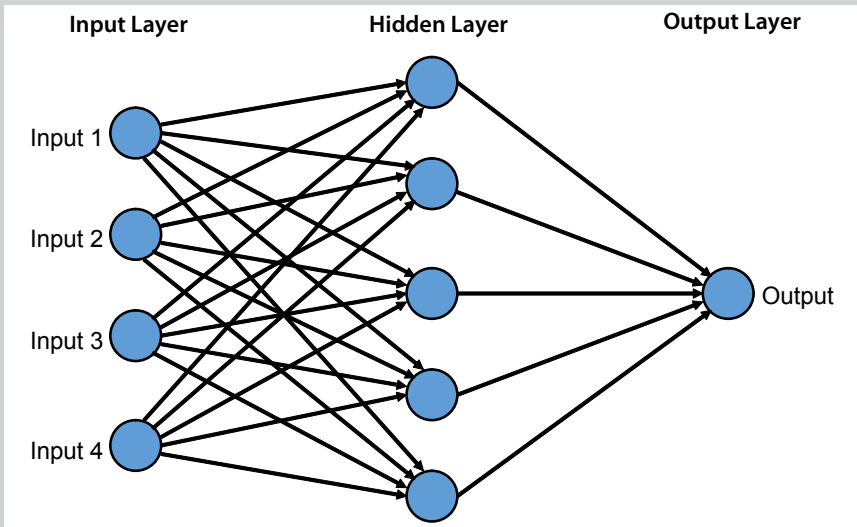


Figure 5: Common architecture of a feed-forward neural network showing highly interconnected nodes akin to the vast network of neurons in a human brain and consisting of three groups of layers: four input units connected to one output unit through five hidden units



reliable experimental data sets, those who develop and apply such models will appreciate the adage, "All models are wrong, but some are useful."

Modern Software Tools: Although process modeling and simulation tools have been available for many years, the first such software products dedicated for bioprocessing showed up in the mid-1990s. Today, several commercially available programs, often using enhanced graphic features and ranging from dedicated packages to fully integrated suites, even include advanced NN and FL technologies (Table 4). Many process simulation software packages also incorporate data mining and analysis features either to find underlying relevant relationships within culture data matrices (featuring both on- and off-line data from process variables) or to handle data sets that may be corrupted by noise or missing data points altogether. Although neural networks are noise tolerant, it is accepted that the best approach for analysis is to identify and remove outlying data points (either discarding such data or converting outliers into missing values) by using statistical tools.

Scale-down Modeling

Mathematically based simulations have much value in process design, optimization, and operation, but much original data must still be obtained in real-world experimentation. Because doing so in actual production environments is too expensive and time-consuming, it is often accomplished in scale-down

bioreactors (2–5 L) that imitate the conditions of full-scale production reactors (≥1,000 L). A first principle in scale-down design is that often the best small-scale conditions must be sacrificed in favor of approximating those that actually can be achieved in a large-scale system. Development of scale-down bioreactor models requires evaluating two distinct sets of parameters: bioreactor design criteria (design parameters) and actual culture operating parameters (process variables).

Unfortunately, no (stirred-tank) bioreactor scale-down design criteria are universally applicable. Not all design parameters can be maintained as identical between large and small scales: e.g., volumetric oxygen transfer coefficient (K_La), shear rate (a function of the impeller tip speed), and impeller pumping capacity (or flow). A scale-down model using a culture vessel of geometrically similar scale can be based on

- equal gassed power input per volume
- equal K_La
- equal shear rate
- equal mixing time
- or a combination of oxygen transfer rate (OTR), shear rate, and mixing parameters.

That final option recognizes that two or more parameters may be of comparable importance, e.g., shear rate and oxygen transfer rate (itself a function of K_La and dissolved oxygen concentration). Conservation of the hydrodynamic behavior between scale-down and production reactors has been a concern for some time, but it is much more attainable now than ever before with the use of an up-and-coming technique known as computational fluid dynamics (CFD) (**59, 60**). CFD software packages (e.g., www.fluent.com, www.cd-adapco.com) illustrate and calculate velocity profiles within culture fluid (across a reactor). They are very effective in assessing mixing characteristics (shear rate, shear stress, and mixing time), gas hold-up, gas dispersion, mass transfer, and even nutrient addition gradients.

Foundations in Bioreactor Process Modeling, continued

Neural networks deploy "hidden" units to conceptualize model parameters that are not directly accessible, which explains the "black-box" nature of the model. The activity of each hidden unit and output unit is determined by both the activities of the previous layer and the weighting of the interconnections. Those interconnections determine whether it is possible for one unit to influence others, and scalar weights specify the strength of such influence.

Because neural networks cannot be programmed, they must be "taught" to perform each particular task by presenting the network with training examples using historical process data obtained from previous culture runs. Such examples consist of a pattern of activities for the input units together with a desired pattern of activities for the output units. To reduce the error between desired and actual outputs, the scalar weight of each connection is adjusted using, for example, the popular back propagation algorithm. Neural networks "learn" by example similar to the way biological systems learn by adjusting the inhibition and excitation effectiveness of synaptic connections between neurons. Because the network discovers how to solve a problem by itself, its operation can be unpredictable. However, an appropriately trained neural network can be thought of as an "expert" capable of analyzing data and making estimations of process values.

The most commonly used NN architectures for process modeling and control are the feed-forward neural network (which allows signals to travel from input to output only) and the recurrent network (which can have signals traveling in both directions by introducing loops with an implied temporal dependence). One difficulty with recurrent networks is determination of the best network architecture with respect to the number of hidden units.

Other proposed advanced network architectures include dynamic, fuzzy, and stacked neural networks. A dynamic neural network (DNN) adapts the static feed-forward network concept by using past process inputs and outputs to predict currently appropriate process outputs.

Because the number of process variables and data are often limited, neuro-fuzzy networks combine fuzzy logic and neural network technology allowing "expert rules" to be added to data sets for improving overall model robustness. That can be very useful in bioreactor processes where controlled variables are often restricted to a limited range for design reasons (e.g., minimum or maximum achievable feed rates) or safety reasons (e.g., maximum allowable liquid volume height, vessel pressure, and so on). Capturing real-life experiences from skilled operators (expert knowledge) for a problem domain augmented with a fair dose of common sense can compensate for sparse and noisy data, often resulting in a faster learning phase. Stacked neural networks have been proposed to further enhance model accuracy and robustness by aggregating several different networks, the output of which are determined by weighing each individual network output against the others for a final consensus.

Statistical Models: A statistical approach is often required because of uncertainties surrounding some process variables. According to some, this is the only true measure of process uncertainty (compared with fuzzy reasoning). Probabilistic models are characterized by probability density functions of the process variables involved, with normal distribution being the most commonly used. Correlation models quantify the degree of similarity between two variables by monitoring their variations. System dynamics are not captured by statistical methods per se, but they play an important role in data mining and analysis, data compression, principal component analysis (PCA), and statistical process control (SPC).

Bioreactors include many accessories (e.g., spargers, probes, and dip tubes) that are added to the basic vessel structure. But their overall influence upon hydrodynamic behavior appears inconsequential for large-scale units. That may not be so, however, for small-scale bioreactor models because of limitations in miniaturizing such accessories. So for scale-down models using conventional stirred-tank technology, the smallest practical volume is about a liter. The first step is always identifying critical design parameters that will provide acceptable criteria upon scale-down.

For a proposed scale-down bioreactor model to meaningfully

Table 4: A nonexhaustive list of commercially available software for data mining and analysis, bioreactor process modeling and simulation, and beyond

Software	Technology	Applications	Reference
Aspen One	Genetic algorithm, hybrid neural network, fuzzy logic and linearized rigorous models	Comprehensive suite for process industries; recipe-based process modeling, analysis, simulation, inferential sensors, scale-up, and optimization	www.aspentech.com
dataEngine	Statistical methods combined with neural network and fuzzy logic	Data analysis and soft computing for process industries; integrates into LabView brand or other existing control systems	www.mitgmbh.de
Desire/Neunet	Neural networks and fuzzy logic	Interactive modeling and simulation of dynamic systems	http://members.aol.com/gatmkorn
G2 NeuOn-Line	Neural network	Comprehensive suite for process industries (data mining, modeling, sensor evaluation, prediction, optimization and control)	www.gensym.com
Lucullus	Neural fuzzy network	Simulation and modeling as part of a process information management system	www.biospectra.ch
Neuframe	Neural network and fuzzy logic	Simulation and process control with expert rules	www.neusciences.com
Neunet Pro	Neural network	Data mining, modeling and prediction	www.cormactech.com
NeuroGenetic Optimzer	Neural network	Older well established data modeling tool	www.bio-comp.com
NeuroModel GenOpt	Neural network and genetic algorithms	Data mining, modeling, plant and process analysis, simulation and optimization	www.atlan-tec.com
NeuroSolutions	Neural network, fuzzy logic, and genetic algorithms	Various neural networks for data mining and modeling	www.nd.com
Matlab and Simulink	Model-based design for physical system behavior	Popular multipurpose platform for simulation and control with particular appeal to engineers.	www.mathworks.com
Statistica	Statistical process control combined with neural network, and clustering algorithms	Data mining, process monitoring, predictive modeling, and visualization	www.statsoft.com
SuperPro Designer	Material and energy balances with comprehensive resource database	Integrated platform for process development, manufacturing process modeling, equipment sizing, evaluation, scheduling, economics and optimization	www.intelligen.com
Viscovery SOMine	Self organizing maps, clustering techniques, and correlation compensation	Data mining, modeling, analysis and visualization	www.eudaptics.de

represent performance for a particular large-scale culture process, a systematic validation study must be conducted. Key process variables need to be identified, e.g., mixing, temperature, pH, dissolved oxygen (DO) and dissolved carbon dioxide (DCO_2). Their relationships with bioreactor performance (such as cell density and viability, product quality and yield, substrate feeding, and waste product accumulation) must be understood through comparisons with profiles from the large-scale process. Those relationships are then characterized as fully as possible and the operating ranges for their respective variables determined.

Particular scale-down modeling challenges arise when the assumption no longer holds true of a well-mixed homogenous bioreactor, without gas-liquid diffusion limitations. Poor mixing can lead to substrate and pH gradients, which are in some cases amplified by addition of concentrated nutrients and reagents at the liquid surface. Inadequate mixing also promotes DO fluctuations because of gas–liquid transfer limitations. Those phenomena were first described in large-scale production reactors used in industrial microbiology settings (**61**) where broth rheology is often non-Newtonian — but they may also apply to ultrahigh-density animal cell culture systems. In such cases, multicompartment model systems (e.g., a stirred-tank reactor integrated with a plug flow reactor) have been proposed to model and account for spatial fluctuations (**62–64**).

There is a continuing desire to decrease culture volumes (and consequently culture populations) to maximize throughput for cell-line screening, media optimization, and process development in fully controlled "miniature" bioreactor systems (<100 mL) with conservation of full predictive model power for scaled bioreactors. That has spurred significant research activities in both industry and academia (**65–68**). Such efforts are likely to challenge the boundaries of our understanding of cellular microenvironments — especially with the advent of future miniaturization down to the "micro" level (<1 mL), which will probably require entirely new approaches to modeling and validation.

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