Getting the Most from Your Bioreactor

by Christian Julien and William Whitford

The earliest example of bioproduction control dates back thousands of years to the ancient Egyptians who created a defined and multistep process for fermentations. By first cooking a batch of malt and wheat, and then adding yeast and uncooked malt to the cooled intermediate product that resulted, they produced suitable media to support the fermentation of a palatable beer (**1**).

In the modern world, the essential operational parameters (process conditions) controlled in basic suspension cell culture include

- maintaining a constant temperature — by control of incubator air
- keeping cells suspended — by agitation of the flask
- providing a single bolus of fresh culture media — at culture seeding
- providing a constant rate of oxygen delivery through the air–culture surface — by exposure to incubator air.

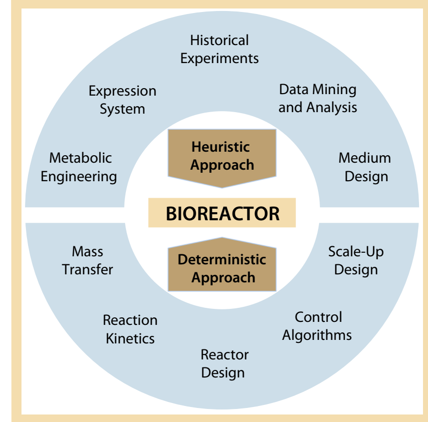
Until recently, this basic system was all that was available to cell culturists. But today's large-scale bioproduction demand for higher productivities, process robustness and reproducibility, transferability, and regulatory compliance has driven a revolution in the number of parameters addressed and the means of their monitoring as well as in the algorithms and hardware used for their control.

Cell-based bioproduction comprise two quite disparate systems: an algorithm-driven mechanical device (the bioreactor) and a biological component (cells in dynamic culture). Optimization of such a system therefore involves a functional interplay of both.

The Process Control Paradigm

The Relationship Between Biology and Mechanics: Modern bioproduction systems would not be possible without our knowledge of cellular functions,

Figure 1: Heuristic and deterministic approaches practiced by cell culturists and process engineers, respectively, to develop bioreactor processes and their control.



biochemical conversions, and metabolic pathways. Even rDNA technology applications have gone beyond a means of introducing exogenous product to the control of cellular activities by manipulating, e.g., cell cycles (**2**) and cellular processes such as glycosylation pathways (**3, 4**).

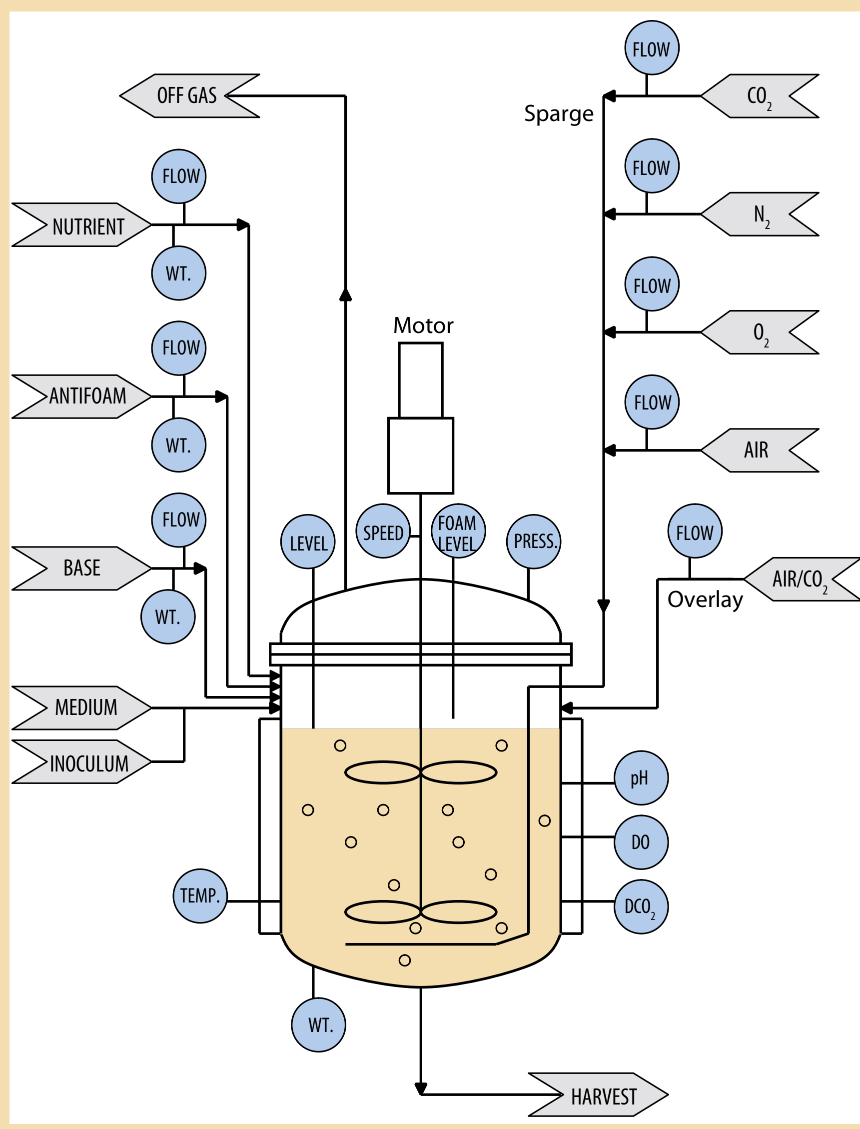
Cell culturists' goals are achieved mainly by enhancing cellular activities and focus on biomass. Whereas biologists have evolved to operate in such advanced fields as metabolic engineering (**5, 6**), these scientists tend to view a physical bioreactor from the top down, as a population of living cells dynamically responding to culture conditions (Figure 1). They approach its control heuristically: by discovering a particular clone and the culture environment (predominantly cellular nutrition) that promotes optimal response from the constituent cells, even though such activities now involve such modern statistical tools as DOE, factorial design approaches (**7, 8**) and data mining. Those methods incorporate information from genomic, proteomic, and metabolomic databases to take advantage of recent developments for model-based experiment medium design and metabolic engineering.

On the other hand, process engineers focus on the equipment and tend to view the bioreactor from the bottom up (Figure 1) as a chemical reactor harboring a set of (admittedly complex) chemical reactions (**9**). So engineers take an overall computational approach and view control in terms of the stoichiometry, thermodynamics, mass transfer, and kinetics of even the most elemental reactions of such components as carbon or total available electrons. Process engineers are driven more deterministically than biologists, and they believe in maximizing process efficiency by focusing on controlling the drivers of chemical reactions within bioreactors.

Neither approach is sufficient, on its own, to provide for efficient development of optimal production conditions. Both provide important aspects of the total bioproduction technology. In a sense, these disciplines are linguistically entangled: We could easily envision that the true process is in fact a set of individual cellular "bioreactors," all operating within a large, mechanical bioreactor. The control challenge — to positively influence each cell's internal environment by manipulating the external environment in which they all live — is best accomplished by combining the knowledge base of both cell biology and engineering. Thus, development timelines may be shortened by facilitating the design of bioreactors that are instrumented and operated to provide more robust control strategies, thereby ensuring more efficient and scalable biomanufacturing processes (**10**). A holistic view of systematically integrating cell biology, micro- and macrokinetics, fluid dynamics (**11**), and thermodynamics (**12**) may be our best overall strategy to enhance bioreactor performance. This has already been emphasized for microbial processes (**13–15**) and should hold equal value for cell culture processes.

Individuals and Populations: Whereas the general biochemical pathways operating within a culture have been well described, each particular cell within that culture can be thought of as an individual unit of production. Currently we can practically monitor, model, and control only what the entire population is doing (on average) — and not what each individual cell's activity is at any given

Figure 2: The well-instrumented industrial animal cell culture bioreactor (for clarity, all actual control elements have been omitted); complete instrumentation may be unnecessary depending on the application, but it provides a good basis for flexibility, especially toward gassing and feeding control strategies; liquid flow and weight measurements are redundant and interchangeable depending on the control approach (Wt. = weight, Temp. = temperature)



time in a process. This has given us two fundamental approaches to the modeling and control of reactors, respectively known as structured–segregated (considering each cell's activity) and unstructured–unsegregated (looking at each chemical reaction in general) (Table 1). Unstructured–segregated modeling, as an intermediate approach, could be conceived of as

- segregating subpopulations based on statistical behavior (**16**) such as to describe the plasmid complement distribution in recombinant cells for assessment of plasmid and culture stability (**17**)
- or segregating subpopulations in transient states (e.g., growth phase segregated from viral infection phase) or physical compartments (e.g., to cope with oxygen gradients in large-scale bioreactors).

A manufacturer's determination of which reactor parameters to (ideally) measure and control is driven by a number of distinct goals, from culture progression to productivity to regulatory compliance. But what is possible to measure and/or control in a particular operation is determined by the style (or geometry) of the reactor and the technology currently available as well as economic, regulatory, and procedural constraints.

The essential purpose of process monitoring and control is to ascertain whether a process is following its required course (monitoring) and to reproducibly direct the adjustment of process parameters (control). This helps us achieve such goals as maximizing product titer and answers such questions as when to terminate the process and/or transfer or harvest its product.

Table 1: Overall classification of process modeling approaches using empirically or scientifically derived theorems that form a basis for qualitative and quantitative understanding of bioreactor processes

Classification of Theorems	Definition	Advantage or Disadvantage
Structured–segregated modeling	Viewing each cell's activity individually, and the net reaction as a sum of these distinct parts	If accurately effected, best reflects the reality of the cell culture system
Segregation in metabolic compartments	Viewing total cell mass in several distinct metabolic compartments by using descriptions of the most important metabolic pathways	Useful for simulations but often not practical for process control due to computational complexity
Unstructured–segregated modeling	Viewing subpopulations of cell's activities, and the net reactions as a sum of the subpopulations	Circumvents problems where entire population is poorly described based on average behavior
Gaussian and binomial distributions	Viewing subpopulations based on stochastic biological events (behavior of the total population is a composite of the subpopulations)	Useful for processes with two underlying statistical partitioning mechanisms
Segregation in transient and physical compartments	Viewing subpopulations in discrete time or spatial boundaries (behavior of the total population is a composite of the subpopulations)	Ability to address discrete culture phenomena or to account for spatial differences in bioreactors
Unstructured–unsegregated modeling	Viewing the reaction process as a whole, or as an average of representative parts	An easier, practical, efficient approximation
Monod and Michaelis–Menton kinetics; Blackman, Tessier, and Moser equations	Early generalizations, with defined parameters of substrate-limited culture expansion	An easy, very unstructured, way of beginning to describe the process
Mass and energy balances	Mass and energy conservation principles applied, but not limited, to substrate and product, in closed system	Requires knowledge or assumptions about overall reaction stoichiometry and thermodynamics
Gas–liquid film theorem	Kinetic model describing interfacial mass transfer such as oxygen transfer rate from gas phase to liquid phase (medium)	Assumes well-mixed culture and plug gas flow; generally employed in calculations for oxygen mass transfer

Process Variables and Culture Monitoring

The Well-Instrumented Bioreactor: Bioprocessing professionals are familiar with the basic monitoring instrumentation of small-scale commercial bioreactors that continuously report such culture process values as dissolved oxygen (DO), pH, agitator speed, and temperature. Figure 2 introduces the concept of a well-instrumented industrial cell culture bioreactor to demonstrate the flexibility and associated complexity of modern designs and process monitoring capabilities. The figure depicts essentially all physical and chemical process parameters that can be readily and reliably monitored using established sensor technology. It also implies a level of flexibility in gas blending and feeding strategies, which are at the core of commercial culture processes but often considered proprietary by biopharmaceutical manufacturers. Table 2 elaborates on this *well-instrumented* concept by providing information on typical value ranges and monitoring devices for certain process parameters. Those provide a basis for optimized configurations that support individual commercial processes.

Common Laboratory Techniques: Many culturists use off-line procedures that provide data on the state of cells in culture (such as viability and density) and their medium (e.g., substrate and product concentrations) as well as the physical environment within the bioreactor itself. One example is daily sampling and measurement of secreted product accumulation using such analytical techniques as ELISA and HPLC. Table 3 provides an overview of typically monitored chemical, biochemical, and biological parameters, with corresponding conventional measurement techniques of interest, all of which are detailed in Chapter 3.

Many of those analytical techniques are conducted off-line in well-equipped QC laboratories to provide essential information on process parameters that cannot yet be obtained directly from the bioreactor. Such information is not only critical to monitoring processes, but has found its

Table 2: Physical and chemical process parameters measured for a well-instrumented industrial animal cell culture bioreactor

Process Parameter	Typical Range	Measuring Devices
Temperature	121–130 °C SIP 35–37 °C growth 2–8 °C cooling	RTD
Agitation speed	Varies with bioreactor size and culture protocol	Frequency feedback encoder/ tachometer
Pressure	1–15 psig	Pressure sensor
pH	6.8–7.8	pH electrode
DO	20–50%	DO electrode
DCO_2	5–25% (40–180 mm Hg)	DCO_2 electrode
Air flow	0.001–0.05 vvm air	Thermal mass flow meter
Gas mix	0.001–0.05 vvm air 0.001–0.05 vvm O_2 0.001–0.01 vvm N_2	Thermal mass flow meters
Gas ratio	0.001–0.05 vvm air 0.001–0.05 vvm O_2	Thermal mass flow meters
Foam level	Varies with bioreactor size and culture protocol	Conductance or capacitance probe
Level	Varies with bioreactor size and culture protocol	Conductance or capacitance probe, d/p cells, or load cells
Flow	Varies with bioreactor size and culture protocol	Magnetic meter, Coriolis mass flow meter, or tachometer (metering pump)
Weight	Varies with bioreactor size	Load cell, balance platform, or d/p cell

d/p = differential pressure cell
DCO_2 = dissolved carbon dioxide
DO = dissolved oxygen
RTD = resistance temperature device
SIP = sterilizable-in-place
vvm = volume of gas per volume of liquid per minute

Table 3: Typical biological, physical and biochemical process parameters measured in animal cell culture — and the conventional laboratory techniques used to do so

Process Parameter	Typical Operating Range	Conventional Laboratory Techniques
Total cell count	0.5–20 × 10^6 cells/mL	Hemocytomer/microscope or automatic cell counter
Viable cell count	0.5–20 × 10^6 cells/mL	Hemocytomer/microscope using trypan blue exclusion
Cell size/volume	10–20 µm	Automatic cell counter
Osmotic pressure	300–350 mOsm/kg	Osmometer
DCO_2	<200 mm Hg	Chemical analyzer
Heat of respiration	Variable	Calorimetry
Glucose	0–25 mmol/L (0–4.5 g/L)	Enzymatic test kit or biochemical analyzer
Glutamine	0–8 mmol/L (0–1.2 g/L)	Enzymatic test kit or biochemical analyzer
Lactate	0–10 mmol/L (0–0.9 g/L)	Enzymatic test kit or biochemical analyzer
Ammonia	0–13 mmol/L (0–0.2 g/L)	Enzymatic test kit or biochemical analyzer
Amino acids (specific)	0–10 mmol/L (each)	HPLC/MS
Protein (specific)	0–10 g/L	ELISA, HPLC/MS, CE, SDS PAGE
NAD/NAD(P)H	0–10 µmol (extracellular) 5 µmol to 5 mmol (intracellular)	Spectrophotometric assay, HPLC, agarose GE, CE
DNA (supernatant)	0–10 mg/L	Spectrophotometric assay, HPLC, agarose GE, CE

CE: capillary electrophoresis
ELISA: enzyme-linked immunosorbant assay
HPLC: high performance liquid chromatography
MS: mass spectrometry
PAGE: polyacrylamide gel electrophoresis

way into supervisory control strategies (and, recently, many expert systems) for advanced control implementations, which will be detailed in Chapter 2.

The Onset of System Integration: Process engineers, on the other hand, have expanded their monitoring arsenal with advanced analytical instrumentation such as off-gas analyzers to measure carbon dioxide and oxygen in bioreactor vent gas, spectroscopy-based analyzers (e.g., mass and infrared), and various chemical and biochemical analyzers. Direct on-line system configurations are preferred. Early examples of process integration consisted of combining off-gas analysis using paramagnetic (for exhaust oxygen), infrared analyzers (for exhaust carbon dioxide), and mass spectrometry (MS), with internal dissolved gas measurements for O_2 and CO_2 (**18**). Those allowed for easy calculation of derived parameters such as oxygen uptake rate (OUR) and carbon dioxide evolution rate (CER) to estimate the specific growth rate. That provided insights in the culture process that spurred new feeding control strategies (**19–21**). Further aspects of system integration will be discussed in Chapter 3.

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