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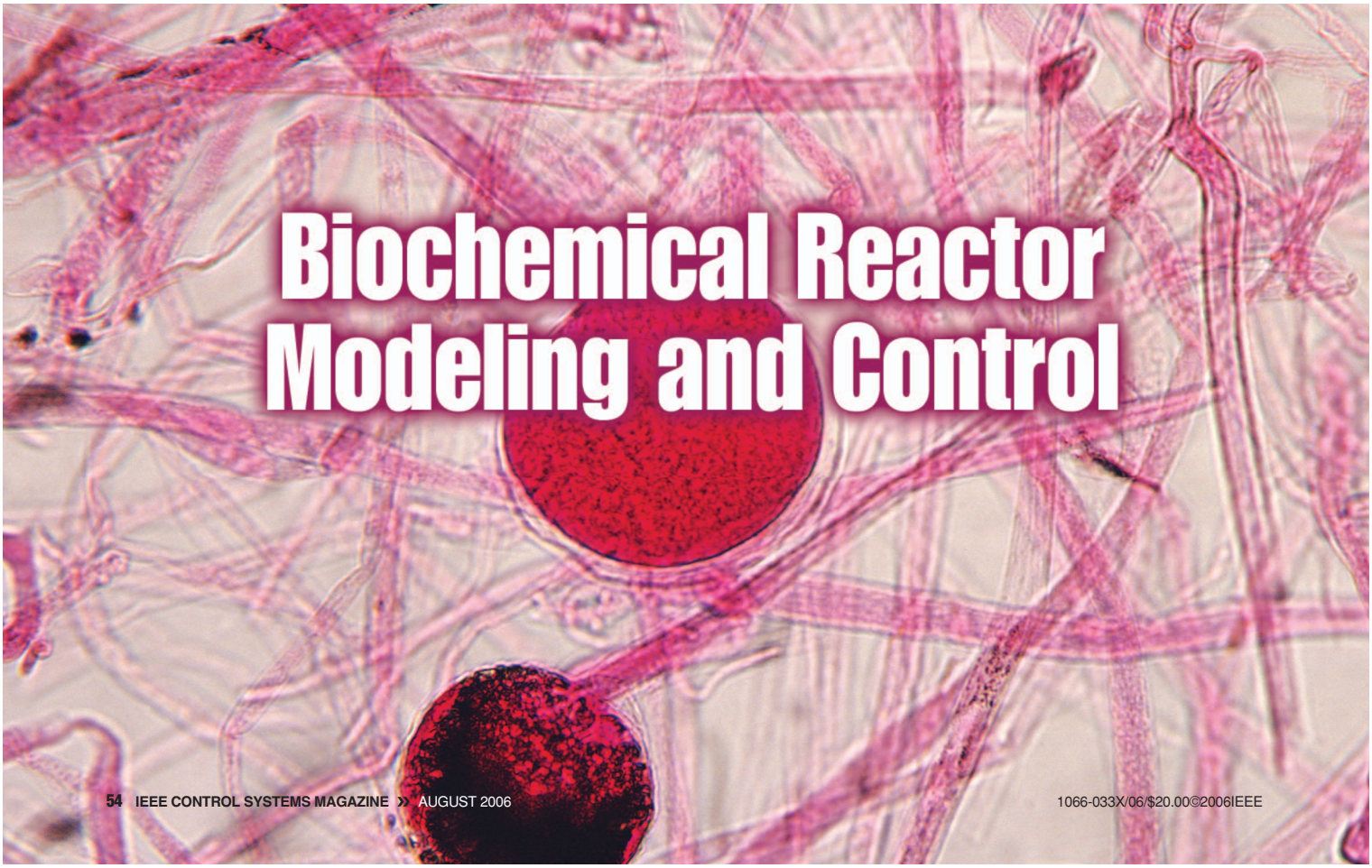
EXPLOITING CELLULAR BIOLOGY TO MANUFACTURE HIGH-VALUE PRODUCTS

The biotechnology industry is expanding rapidly due to advances in understanding complex biological systems and the high demand for biologically manufactured products such as foods and beverages, pharmaceuticals, and commodity and specialty chemicals. The impact of the biotechnology industry on the global economy is substantial. The revenues of the top ten U.S. pharmaceutical manufacturers totaled US$217 billion in 2002 with profits of US$36 billion [1]. A growing biotechnology market is the large scale production of ethanol as a renewable liquid fuel. The production of ethanol in 1998 was 31.2 billion liters worldwide and 6.4 billion liters in the United States with roughly two-thirds of the ethanol produced targeted for biofuel applications [2].

A typical biochemical manufacturing process consists of a reaction step in which a large number of cells are used to synthesize the desired product followed by a series of separation steps, in which the product is recovered from constituents of the reaction liquid. The key requirement for the manufacturing process is the identification of a cell type that converts a relatively inexpensive chemical species to the desired biochemical product. Advances in recombinant DNA technology facilitate the design of genetically engineered cell strains to enhance the yield of a target product [3]. While most industrial processes are based on microbial cells such as bacteria and yeasts, other cell types obtained from plants and animals are increasingly being utilized to produce high-value pharmaceutical products such as therapeutic proteins [4].

Because each cell produces only a miniscule yield of a given product, a large number of cells are needed to obtain commercially viable production rates. The cells are grown and products are harvested in large vessels known as biochemical reactors or fermenters. Penicillin production was revolutionized by the combination of genetically engineered cell strains and large-scale fermentation, as illustrated in "Penicillin Production." The liquid removed from the reactor contains a mixture of biochemical species that must be separated to recover the desired product. The recovery step is usually achieved through a series of separation units [5]. The development of process control strategies for these separation systems is an important research problem.

Process control has played a limited role in the biotechnology industry as compared to the petroleum and chemical industries. This demand for process modeling and control is increasing, however, due to the expiration of



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pharmaceutical patents and the continuing development of global competition in biochemical manufacturing. The lack of online sensors that allow real-time monitoring of the process state has been an obstruction to biochemical process control. Recent advances in biochemical measurement technology, however, have enabled the development of advanced process control systems.

BIOCHEMICAL REACTOR TECHNOLOGY

Basic Operating Principles

A schematic representation of a biochemical reactor (bioreactor) is shown in Figure 1. The cells are innoculated in the bioreactor to initiate cell growth. Inoculation occurs through a multistep procedure in which cells grown in a shake flask are transferred to increasingly larger bioreactors. This procedure is necessary for achieving sufficiently large density ($\sim 10^{13}$ cells/l) to sustain growth. The cells are continuously fed a liquid medium stream containing chemicals that act as carbon, nitrogen, and phosphorous sources as well as other components including salts, minerals, and vitamins that replicate the natural growth environment. These chemicals serve as the nutrients, which are also called substrates. Careful preparation of the medium is essential since most cells are sensitive to changes in their growth environment. In aerobic operation, the cells utilize oxygen as a substrate, and air must be continuously supplied to maintain the necessary dissolved oxygen concentration. By contrast, anaerobic operation does not require oxygen to achieve cell growth and product formation. Usually the medium is prepared such that a single nutrient such as glucose limits growth. This nutrient is thus a growth-limiting substrate.

An agitator is used to continuously mix the liquid contents, thereby minimizing spatial gradients in substrate concentrations and cell density, which can reduce bioreactor productivity. The agitator speed is chosen to provide adequate mixing while avoiding excessive shear forces that can rupture the cells. In Figure 1, a stream is continuously removed from the reactor to achieve constant liquid-volume operation. The liquid removal rate is characterized by the dilution rate, which is the ratio of the volumetric

Penicillin Production

Penicillin can be considered the first biotechnological product produced on a large scale with bioreactor technology. In 1929 the antibiotic capabilities of penicillin were discovered by Alexander Fleming at St. Mary's Hospital in London using simple Petri dish experiments in which bacterial colony growth was prevented by the application of penicillin produced by the mold *Penicillium notatum*. Twelve years later, during World War II (WWII), research and development efforts focused on large-scale penicillin production to reduce the devastating effects of infections in wounded soldiers. The two landmarks that allowed rapid achievement of this goal were the discovery of *Penicillium* strains that achieved substantially higher penicillin production rates and the development of bioreactor technology for large-scale growth of *Penicillium* populations [35].

As part of an extensive effort to identify an improved penicillin-producing strain, in 1942 researchers in the United States fortuitously discovered that the mold *Penicillium chrysogenum* obtained from a supermarket cantaloupe in Peoria, Illinois, yielded approximately 200 times more penicillin than *Penicillium notatum*. Further enhancements in penicillin production were achieved by introducing random genetic mutations into this *Penicillium chrysogenum* strain through the application of X-ray and ultraviolet irradiation. This primitive form of genetic engineering ultimately yielded *Penicillium chrysogenum* strains that produced approximately 1,000 times more penicillin than the original *Penicillium notatum* cultures. These strains remain the basis for commercial penicillin production [35].

Despite these advancements, the vast amount of penicillin required for the war effort was seriously impeded by the lack of large-scale production technology. Oxford researchers had shown that *Penicillium* could be grown in nutrient-rich broths introduced into small shallow containers. However, hundreds of these small containers were needed to produce enough penicillin for a single individual. Subsequent research in the United States demonstrated that substantially enhanced production could be achieved by growing *Penicillium* cells in large "deep fermentation" tanks with liquid volumes of 25,000 gal. These bioreactors were continuously supplied with purified air and agitated to allow aerobic growth throughout the tank rather than just on the liquid surface as with previous technology. Further production enhancements were realized when corn steep liquor, a waste material of corn processing, was utilized as a cheap source of concentrated nutrients.

These developments combined to make large-scale penicillin production a reality [35]. By the end of WWII, pharmaceutical companies were producing enough penicillin to treat 7 million patients per year. As a result, the death rate from pneumonia in the U.S. Army dropped from 18% in World War I to less than 1% by the end of WWII. Penicillin is now the most widely prescribed antibiotic for illnesses ranging from strep throat to venereal diseases. The bacterial diversity that allowed the discovery of highly productive *Penicillium* strains has the unfortunate consequence of promoting mutations that produce bacteria resistant to penicillin and other antibiotics. For this reason, advances in production technology must be accompanied by efforts to discover new antibiotics.

feed flow rate to the liquid volume. The effluent stream contains unconsumed media components, cellular biomass, and products excreted by the cells. The desired product, consisting of the cells themselves or a product of cell metabolism such as ethanol, is separated from the other components by a series of recovery and purification operations. Off-gases such as carbon dioxide are also generated as byproducts of cell metabolism.

Effective operation of an industrial bioreactor requires not only supplying the necessary nutrients and extracting the desired products but also maintaining the sterility of the medium and processing equipment. A miniscule amount of microbial contamination can lead to production of a foreign microbe rather than the desired microbe, resulting in complete loss of productivity and an unscheduled shutdown of the bioreactor. Process control plays an important role in maintaining an environment that facilitates cell growth and product synthesis. Each cell type has a unique and narrow range of temperature and pH that supports cell growth. Most bioreactors use simple proportional-integral-derivative (PID) feedback control loops to maintain the liquid temperature and pH at predetermined setpoints.

A feature of bioreactors is their unusually slow dynamics, which are characterized by the residence time (inverse of the dilution rate) for continuous operation. A typical dilution rate of 0.2 h^{-1} is equivalent to an open-loop time constant of 5 h. The slow speed of these dynamics has important implications for control system design. In the United States, all aspects of biochemical manufacturing processes are subject to stringent validation mandated by the Food and Drug Administration. These requirements place demands on the process control system to achieve reproducible operating conditions and consistent product quality.

Most industrial bioreactors are operated in batch or fed-batch mode to allow more efficient media utilization and to avoid sterility problems caused by continuous liquid removal. In batch operation, the bioreactor is initially charged with cells and medium containing essential substrates for growth. The bioreactor then evolves to a predetermined final time with no media feed or liquid withdrawal. Fed-batch operation differs from batch operation in that fresh media feed is continuously supplied. Because there is no liquid withdrawal, the reactor volume increases until the final batch time. An advantage of fed-batch operation is that nutrient levels are continuously varied to achieve favorable growth conditions without significant risk of culture contamination. As discussed below, the fed-batch mode involves dynamic optimization of the bioreactor operating conditions.

COMMERCIAL EQUIPMENT

An industrial-scale bioreactor system manufactured by New Brunswick Scientific is shown in Figure 2. This 1,500-l bioreactor, consisting of the cylindrical stainless steel vessel, is equipped with numerous stainless steel tubes, valves, and electronic instruments to manipulate feed-stream and withdrawal-stream flow rates and to monitor growth conditions. The headplate located at the top of the bioreactor and shown in Figure 2(b) has openings to allow the insertion of tubes for feed and withdrawal streams and online probes for measuring temperature, pH, level, and oxygen concentration of the liquid mixture (see Figure 1).

Conventional bioreactor control systems are designed to supply the prescribed flow of nutrients while avoiding growth conditions that adversely affect productivity. PID control loops are used to regulate temperature, pH, and dissolved oxygen concentration. This simple regulatory structure is effective due the availability of cheap, accurate, and reliable sensors for these environmental variables [6] in contrast to physiological variables, such as the growth rate, which provide a more direct measure of the cellular state. With regard to key output variables such as cell density and product concentration, this regulatory structure represents an open-loop control strategy that fails to account for cellular and media variations present

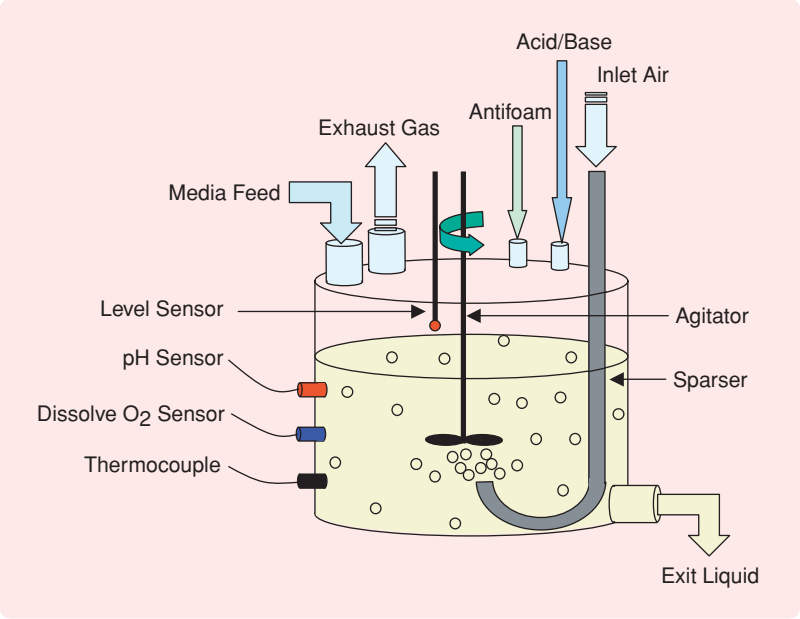

FIGURE 1 Continuous biochemical reactor (bioreactor) for aerobic manufacturing of biological products. A liquid media stream containing nutrients and an air stream containing oxygen are continuously supplied to the bioreactor to sustain cell growth. The liquid level is maintained constant by continuously removing a stream containing unconsumed nutrients, cellular biomass, and products of cellular metabolism. The level, temperature, pH, and oxygen content of the reaction liquid are measured online and used as feedback signals for regulatory control.

in an industrial manufacturing environment. Most physiological measurement techniques are limited to offline analysis in a research laboratory environment [7].

Recent advances in online measurement technology have resulted in model-based control strategies that offer the potential for improved bioreactor performance. Online spectrophotometers are routinely used to measure the cellular biomass concentration [7]. Figure 3 shows commercially available technologies for measuring the compositions of the bioreactor off-gas and liquid-reaction media. The New Brunswick gas analyzer in Figure 3(a) provides real-time measurements of the gas-phase oxygen and carbon dioxide concentrations, which are indicative of the cellular state. Direct information on substrate utilization and product formation rates can be obtained from the biochemical analyzer shown in Figure 2(b). When combined with an automatic sampling system, this analyzer provides simultaneous concentration measurements of two biochemical species, such as glucose and ethanol, every few minutes. Simultaneous measurements of extracellular species are obtained with online gas chromatography and high-performance liquid chromatography [6], [7]. More sophisticated measurement technologies that provide online measurements of intracellular species concentrations and heterogeneities across the cell population are under development [7].

DYNAMIC MODELING OF BIOCHEMICAL REACTORS

Model Complexity

Mathematical modeling of bioreactors is a challenging problem due to the complexity of cellular metabolism. The appropriate degree of model complexity is determined by factors such as the amount of fundamental knowledge, data requirements for model construction and validation, computational requirements, and the intended use of the model. Dynamic bioreactor models are classified according to the level of detail used to describe an individual cell. The most mechanistic descriptions of cellular metabolism are based on structured kinetic models, where the rates of individual enzyme-catalyzed reactions are embedded within dynamic mass balance equations for the intracellular species [8]. Due to experimental difficulties associated with large-scale identification of enzyme kinetics, these ordinary differential equation models are effectively limited to primary metabolic pathways and are not well suited for capturing whole-cell metabolism, which impacts cellular growth and product synthesis rates. As a result, these models have not yet been used for bioreactor control.

Segregrated models account for population heterogeneities by differentiating individual cells according to scalar variables such as cellular mass or DNA content.

(a) (b)

FIGURE 2 Industrial scale bioreactor for manufacturing biological products. The complete bioreactor system shown in (a) includes the 1,500-l reaction vessel and stainless steel tubes, valves, and electronic instruments for flow-rate manipulation and continuous monitoring of growth conditions. The bioreactor headplate in (b) provides openings for inserting feed and withdrawal tubes as well as submerged sensors for measuring properties of the reaction liquid. (Courtesy of New Brunswick Scientific.)

While control strategies based on segregated models have been explored in simulation studies [9], the construction and validation of these partial differential equation models is difficult in practice. Due to their mathematical simplicity, dynamic models based on unstructured descriptions of cellular metabolism and unsegregated representations of the cell population are best suited for model-based controller design [10]. Rather than model individual enzyme-catalyzed reactions, lumped descriptions of cellular metabolism are employed. Cellular heterogeneities are ignored, and the model equations represent the dynamics of an "average" cell. The use of such unstructured models for bioreactor control is the focus of this article.

Continuous Bioreactor Model

A simple anaerobic growth model for a continuous bioreactor consists of the ordinary differential equations [11]

$$\begin{aligned}\frac{dX}{dt} &= -DX + \mu(S,\ P)X,\\ \frac{dS}{dt} &= D(S_f - S) - \mu(S, P)X/Y_{X/S},\\ \frac{dP}{dt} &= -DP + [\alpha\mu(S, P) + \beta]X,\end{aligned}$$

where X is the concentration of the cellular biomass, S is the concentration of the growth limiting substrate such as glucose, P is the concentration of the desired product such as ethanol, S_f is the concentration of the growth limiting substrate in the feed stream, and $D = F/V$ is the dilution rate, where F is the volumetric flow rate of the feed stream and V is the constant liquid volume in the bioreactor. Cellular growth is characterized by the specific growth rate μ. The yield parameter $Y_{X/S}$ represents the cell mass produced from a unit mass of substrate. The parameter α is the inverse of the product yield associated with cellular growth, while β is the inverse of the growth-independent product yield. Although yield coefficients often vary with the environmental conditions, these parameters are usually treated as constants for simplicity.

The overall accuracy of the bioreactor model depends strongly on identification of a growth-rate function that adequately describes cellular growth over the range of environmental conditions. The function

$$\mu(S,\ P) = \frac{\mu_m(1 - P/P_m)S}{K_m + S + S^2/K_i},$$

where μ_m is the maximum growth rate, K_m is the substrate saturation constant, K_i is the substrate inhibition constant, and P_m is the product inhibition constant, is sufficiently general to describe many situations of practical interest [12]. A saturation function is obtained when substrate and product inhibitory effects are negligible in the limit of large K_i and P_m. In this case, the growth rate increases monotonically with substrate concentration, and μ_m represents the maximum growth rate obtained in the limit of infinite substrate

(a) (b)

FIGURE 3 Commercial technology for online measurement of bioreactor growth conditions. The exhaust gas analyzer in (a) provides continuous measurements of oxygen and carbon dioxide concentrations in the vapor space above the reaction liquid. (Courtesy of New Brunswick Scientific.) The biochemical analyzer in (b) provides online concentration measurements of two biochemical species such as glucose and ethanol in the reaction liquid. (Courtesy of YSI Incorporated.)

concentration. The expression for $\mu(S, P)$ given above is needed when high substrate or product concentrations inhibit cellular growth. For example, high concentrations of the product ethanol are known to inhibit yeast growth. Yield and growth-rate parameters are available for well-studied cell types grown under standard conditions. Otherwise, these parameters can be determined from experimental data using offline parameter estimation techniques [4].

The steady-state behavior of the bioreactor model is characterized by two types of equilibrium solutions. The first type corresponds to the undesirable trivial or washout solution

$$\bar{X} = 0,\ \bar{S} = S_f,\ \bar{P} = 0,$$

where the overbar denotes a steady-state solution. Washout occurs when none of the substrate entering the bioreactor is converted to biomass or product. The number of nontrivial steady-state solutions for which the biomass and product concentrations are strictly positive depends on the specific growth rate function. If substrate and product inhibition effects are negligible, then the growth rate is a saturation function that yields a single nontrivial solution. In this case, when the dilution rate D is less than a critical dilution rate D_c that depends on the model parameter values, the nontrivial steady-state solution is stable [13]. Otherwise, for large D the washout steady state is stable because the bioreactor residence time is not sufficiently large to sustain cell growth. Consequently, control system design must consider the tradeoff between stability margin, which requires small D, and high throughput, which requires large D.

The growth rate function $\mu(S, P)$ yields steady-state input multiplicity. For example, each value $Q = DP$ of the productivity can be obtained with two different dilution rate values [12]. The input multiplicity is characterized by a maximum in the productivity versus dilution rate curve, where the steady-state gain changes sign. The presence of a zero steady-state gain poses difficulties for control design when the objective is to stabilize the bioreactor near the point of maximum productivity. The continuous bioreactor model also exhibits a significant degree of open-loop nonlinearity [12]. Figure 4 shows the evolution of the product concentration P from a common initial condition for step changes in the dilution rate D [Figure 4(a)] and the feed substrate concentration S_f [Figure 4(b)]. The larger D steps induce highly asymmetric responses because the washout steady state becomes stable for the positive change. For the small changes of S_f, significant differences in characteristic time constants and steady-state gains are observed. Even more pronounced asymmetries are evident for the large S_f steps, where the positive change induces an inverse response due to the zero gain singularity at the maximum productivity. These strong nonlinearities must be considered in the controller design process [14].

Fed-Batch Bioreactor Model

The bioreactor model equations can be rewritten to describe batch or fed-batch operation by modifying the flow-dependent terms. For fed-batch operation, the model equations are

$$\begin{aligned}
\frac{d(VX)}{dt} &= \mu(S, P)XV,\\
\frac{d(VS)}{dt} &= FS_f - \mu(S, P)XV/Y_{X/S},\\
\frac{d(PV)}{dt} &= [\alpha\mu(S, P) + \beta]XV,\\
\frac{dV}{dt} &= F,
\end{aligned}$$

where the last differential equation models the time-varying, liquid-volume V. Fed-batch bioreactors are operated by initially charging the vessel with medium and pregrown cells, feeding fresh medium as cell growth progresses and then removing the reaction liquid at a

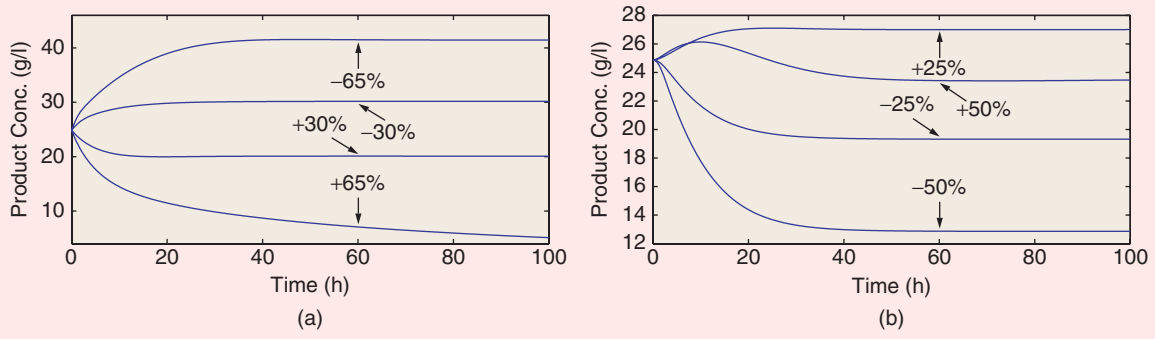

FIGURE 4 Dynamic responses of the continuous bioreactor model for symmetric step changes in (a) the dilution rate D and (b) feed-substrate concentration S_f from a common initial condition. The $\pm 65\%$ D steps induce highly asymmetric responses because the washout steady state becomes stable for the positive change. The large steps in S_f yield more severe asymmetries due to the inverse response for the $+50\%$ change [12].

predetermined time to recover the desired product. Batch processes are operated similarly except that fresh medium is not provided during the batch. Consequently, the concept of a steady-state operating point is not meaningful for batch and fed-batch bioreactors. Instead of designing a feedback controller to stabilize a particular equilibrium point, the prototypical control problem for a fed-batch reactor is to determine a nutrient feeding policy that maximizes the amount of product at the final batch time.

PROCESS CONTROL OF BIOCHEMICAL REACTORS

Control Objectives

Regardless of the bioreactor operating mode, the overarching control objective is to maximize total production of the desired product. Most bioreactors are equipped with sensors for online measurement of the temperature, pH, level, and oxygen concentration of liquids. Simple PID regulatory loops are used to maintain the pH and temperature at constant setpoints predetermined to promote cell growth and product formation. The primary manipulated inputs available for higher level controllers are the nutrient flow rates and concentrations. For the simple case of a single rate-limiting substrate, the available manipulated inputs are the dilution rate D and the feed substrate concentration S_f.

The appropriate strategy for achieving the control objective depends strongly on the operating mode, the availability of additional online measurements, and the accuracy of the dynamic bioreactor model. The most important determinant is the operating mode since continuous operation involves regulation at an equilibrium point whereas fed-batch operation requires computing and tracking dynamic trajectories. The continuous and fed-batch control problems are considered separately below due to their fundamentally different nature. Batch bioreactors are not discussed further since their lack of feed and withdrawal streams does not allow feedback control during the batch. However, run-to-run control strategies have been successfully applied to batch operation [15].

Continuous Biochemical Reactors

A control objective for the bioreactor is regulation at an operating point that maximizes the steady-state productivity. Determination of an appropriate operating point is challenging due to the effect of the environment on cellular metabolism resulting in highly nonlinear behavior (Figure 4). Common industrial practice is to determine the operating point through a time-consuming and expensive experimental design procedure [16]. When a sufficiently accurate bioreactor model is available, the optimal operating point can be determined offline using simple optimization techniques [12]. Online optimization strategies based on adaptive extremum seeking control address the significant errors present in many unstructured bioreactor models [17].

Once the desired operating point is determined, the next step is to design a feedback controller that achieves regulation despite unmeasured disturbances that emanate from sources such as nutrient variations, inadequate liquid mixing, poor pH and temperature control, and time-varying cellular metabolism. Both simple PID and model-based control strategies are used. Controller design is heavily influenced by the online measurements available as feedback signals. Due to their high reliability and low cost, analyzers that provide dissolved oxygen concentration and exhaust gas concentration measurements (Figure 3) are commonly used for control system design [18]. The main limitation of this approach is that oxygen and carbon dioxide concentration measurements provide indirect measures of cellular metabolism, and thus regulation of these measurements at predetermined steady-state values cannot be expected to result in optimal productivity.

The availability of online analyzers that provide direct measurements of substrate and product concentrations has enabled the development of more effective control strategies for continuous bioreactors. Commercial instruments such as the YSI biochemical analyzer (Figure 3) provide these concentration measurements every few minutes, while the time constant of a typical continuous bioreactor is several hours. The rapid analyzer sampling rate allows the controller design problem to be treated in continuous time. Both nonadaptive and adaptive nonlinear control strategies have received attention due to the presence of highly nonlinear and uncertain process dynamics [18]. The continuous bioreactor model is well suited for applying nonlinear control-design methods based on differential geometry [19], and both state-space linearization [20] and input-output linearization [21] techniques have been investigated. Because offset-free regulation of the controlled output at a specified setpoint is required, input-output linearization is usually the preferred method [12].

Feedback linearizing control strategies suffer from practical limitations such as the need for accurate dynamic models of cellular growth and product yields as well as the availability of online measurements of the cellular biomass, substrate, and product concentrations. While reasonably accurate values of the yield coefficients can often be obtained, the determination of a growth rate function that captures cellular metabolism over a wide range of bioreactor operating conditions is notoriously difficult. Adaptive versions of input-output linearization in which the growth rate μ is treated as an unknown, time-varying parameter are experimentally evaluated in [11] and [22]. Although model-based controller implementation is facilitated by recent advances in biochemical measurement technology, industrial manufacturing processes often lack online concentration measurements of the cellular biomass X, the rate-limiting substrate S, or the desired product P. Simple nonlinear state estimators [23], [24] can be combined with adaptive input-output linearizing controllers to yield satisfactory closed-loop performance [22].

Fed-Batch Biochemical Reactors

Because they are operated dynamically over a finite batch time, fed-batch bioreactors offer a unique set of challenges for control system design and analysis. Rather than stabilize a fixed operating point, the control objective is to maximize the amount of product at the final batch time. The productivity depends on the initial batch conditions, the nutrient feeding policy, and the batch duration. Computational methods are required to determine optimal fed-batch control policies because the effects of these variables on cellular metabolism and product formation are complex. The open-loop optimal policy can be determined by solving an optimal control problem [10], [18].

An objective function representing the total mass of the desired product at the final batch time is maximized subject to constraints imposed by the dynamic model and operational limitations. Various computational algorithms are used for dynamic optimization. Sequential solution methods involve iteration between a dynamic simulation code, which integrates the model equations given a candidate feeding policy, and a nonlinear programming code, which processes the dynamic simulation results to determine an improved feeding policy. While they are relatively straightforward to develop, sequential solution methods exhibit slow convergence and occasional failure for large optimization problems.

Simultaneous solution methods, in which model integration and operating policy optimization are embedded within a single computational algorithm, provide a more efficient and robust problem solution [25]. The primary difficulty is that most nonlinear programming codes cannot accommodate differential equation constraints. Simultaneous solution methods based on temporal discretization of the dynamic model equations are effective due to their ability to explicitly account for state-dependent constraints and their applicability to large optimal control problems. The dynamic optimization approach is applied to simulated fed-batch bioreactors in [17] and [26] as well as experimental systems in [27]. A representative problem is the maximization of protein production by manipulating substrate-feed flow rates [28].

Numerical solution of the fed-batch optimization problem yields an open-loop control policy that maximizes productivity. In practice, direct implementation of the open-loop policy yields suboptimal performance due to the presence of structural modeling errors and unanticipated disturbances during the batch. A common approach to handle unmodeled bioreactor dynamics is to combine a feedback controller with an online state estimator to correct the dynamic model predictions when measurement information becomes available [22]. A unique feature of fed-batch operation is the presence of a final batch time such that the time horizon for estimation and control becomes shorter as the batch proceeds. Extensions of model predictive control based on the concept of a shrinking horizon address this class of problems, assuming predetermined initial batch conditions and a fixed final batch time [29].

The shrinking horizon control problem is solved from the current time instant to the final batch time by using the most current state estimate to reset the initial conditions of the bioreactor model. To compensate for modeling errors and disturbances, only the first set of calculated nutrient feed changes is implemented. Then the optimization problem is re-solved at the next time instant over a shorter horizon using the new state estimate. Applications of shrinking horizon control to fed-batch bioreactors include [29], [30].

An alternative class of fed-batch bioreactor control strategies based on regulating a substrate or product concentration at a predetermined setpoint that maximizes the predicted cellular growth rate is investigated in [24], [31]. Figure 5 shows that a simple PID controller can achieve tight glucose regulation in a fed-batch yeast bioreactor despite large variations in the glucose consumption rate.

FUTURE DIRECTIONS

Process control is expected to play an increasingly important role in the biotechnology industry. The development of feedback control systems that exploit advances in online measurement technology to achieve optimal productivity of continuous and fed-batch bioreactors is one of the most important challenges in biochemical manufacturing. This article provides an overview of current and emerging bioreactor control strategies based on unstructured dynamic models of cell growth and product formation. Despite their widespread acceptance, these models suffer from several fundamental limitations, including

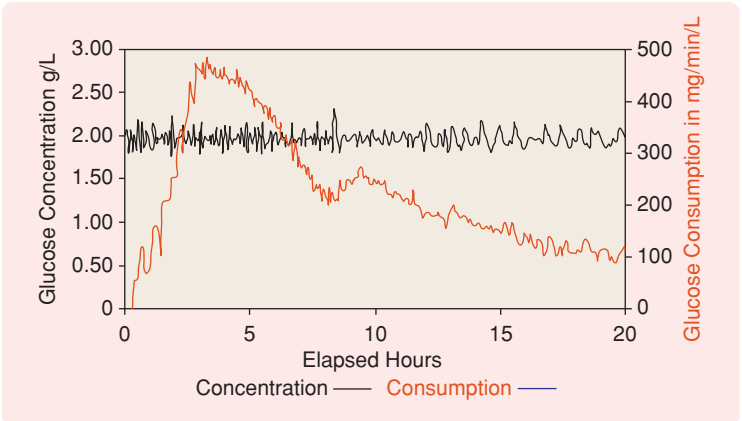

FIGURE 5 PID control of glucose concentration in a fed-batch yeast bioreactor using online measurements from a biochemical analyzer. The glucose concentration is tightly regulated at its setpoint value (2 g/l) despite large variations in the glucose consumption rate. (Courtesy of YSI Incorporated.)

lumped descriptions of cellular metabolism and the assumption of cellular homogeneity. In addition to extending the applicability of existing methods, future research and development efforts are expected to focus on the utilization of more detailed dynamic models for bioreactor optimization and control.

As an alternative to kinetic models that require individual enzyme kinetics, steady-state mass balance equations can be used to describe intracellular reaction pathways under the realistic assumption that intracellular dynamics are much faster than the environmental dynamics. The steady-state intracellular model can be combined with dynamic mass balance equations for key substrates and products to construct a dynamic flux balance model that generates accurate predictions of the growth rate and product yields [32]. These models are currently being utilized to develop optimization and control strategies for fed-batch bioreactors [33]. The recent development of online flow cytometry allows heterogeneities across a cell population to be quantified in real time [34]. Dynamic measurements of DNA and protein content distributions can be used as feedback signals for nonlinear controllers that regulate cell population properties [9].

AUTHOR INFORMATION

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