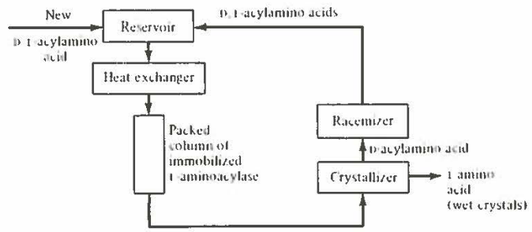

Figure 9P12.1 Catalyzed resolution of amino acids. *[From T. Tosa, T. Mori, N. Fuse, and I. Shibata, Enzymologia, vol. 31, p. 225, 1966.]*

9.12 Optically pure amino acids The process developed by Tosa *et al.* ("Studies on Continuous Enzyme Reactors II. Preparation of DEAE-Cellulose Aminoacylase Columns and Continuous Optical Resolution of Acetyl-d,l-methionine," *Enzymologia*, **31**, 225, 1966) can be represented schematically by Fig. 9P12.1. Assume for the moment that r_{max} is independent of pH. The initial racemic amino acid solution is acetylated by reaction with acetic anhydride; the L-aminoacylase column reverses the acetylation reaction for the L-amino acid, which is then crystallized in alcohol solutions.

(*a*) Assume that the initial amino acid concentration is $5 \cdot K_m$; develop an expression for the fractional L-amino acid conversion achieved by the enzyme column in plug flow. Repeat including axial dispersion.

(*b*) The racemization reaction may be taken to be first order reversible, so that the rate is proportional to $c_{D\,acid} - c^*_{D\,acid}$, where $c^*_{D\,acid}$ is equilibrium D acid level for the solution. If 90 percent of the L acid and 1 percent of the D acid is removed in the wet-crystal stream, along with 10 percent of the entering aqueous phase, what racemization CSTR volume is needed to achieve 95 percent approach to equilibrium?

(*c*) The enzymatic deacetylation step releases acetic acid into the solution. If the pK's leading to enzyme deactivation are $pK_1 = 5$ and $pK_2 = 8$, what entering pH would give maximum conversion for a 10^{-6} *M*, 10^{-4} *M*, or 10^{-2} *M* feed mixture? (Assume plug flow.) State your assumptions clearly.

9.13 Digestion of insoluble substrates As an example of processes involved with digestion of particulate substrates, the following unit-operation sequence for yeast growth on newsprint has been suggested: mechanical grinding, acid hydrolysis, medium neutralization, addition of additional minor nutrients for yeast growth, yeast fermentor (aerobic), vacuum filtration to separate liquid from cell mass.

(*a*) Sketch the flow scheme above, indicating by arrows points of addition and by circles each unit operation. Include solids conveyers and liquid-pump locations where needed.

(*b*) From any human physiology text, sketch the human food-digestion process in a similar manner.

(*c*) Bionics is the study of natural systems with an eye toward development of synthetic analogs. Discuss similarities and differences between processes (*a*) and (*b*). How might you design a solids handling scheme for part (*a*) using the "conveyer" type in part (*b*)?

9.14 Cell maintenance; washout at small S For some populations, a minimum level of substrate may be needed to achieve a nontrivial steady state. As an example, consider the system with kinetics of the form

$$r_s = \frac{\mu_{max} s x}{K_s + s} \qquad k_e x \qquad r_x = \left(\frac{1}{Y}\right)\frac{\mu_{max} s x}{K_s + s}$$

Assuming that the design basis underlying Prob. 9.10 is valid

(*a*) Show that at substrate level below $k_e K_s/(\mu_{max} - k_e)$ the only steady state in a CSTR system is $x = 0$

(*b*) If $\mu_{max} = 0.5\ h^{-1}$, $K_s = 0.2\ g/L$, $k_e = 0.1\ h^{-1}$, and $Y_x = 0.6$ g cell/g substrate, plot $(dx/dt)_{batch}$ vs. x and prove by direct solution of the above equations that $dx/dt < 0$ for low x and for $D > D$ (washout).

(*c*) Determine the stability of each steady state to small perturbations.

9.15 Whey fermentation The fermentation of whey lactose to lactic acid by *Lactobacillus bulgaricus* at 44°C and pH 5.6 has been observed to fit the model of Luedeking and Piret [Eq (7.93)] provided the following modifications are made:

1. The maximal growth rate is $\mu_{max}\left(1 - \frac{p}{p_{max}}\right)$
2. $\mu_{max} = 0.48\ h^{-1}$, $p_{max} = 5.0\%$ at $p \leq 3.8\%$
   $\mu_{max} = 1.1\ h^{-1}$, $p_{max} = 4.3\%$ at $p > 3.8\%$
3. Parameters for continuous fermentation are: $\alpha = 2.2$
   $\beta = 0.2\ h^{-1}$
   $Y = 0.88$ g product/g substrate
   $K_s = 50$ mg/L

(*a*) Write down the equations for s, x, and p in continuous fermentation.

(*b*) Assuming steady-state behavior, show that at a total retention time of 15 h, two equal stages are better than one, but three produce essentially no further improvement in the reduction of substrate level. Is the same result true for biomass? (Consider the case $s_0 = 5.0\%$, $x_0 = p_0 = 0$).

(*c*) Keller and Gerhardt ("Continuous Lactic Acid Fermentation of Whey to Produce a Ruminant Feed Supplement High in Crude Protein" *Biotech. Bioeng.*, **17**, 997, 1975.) note that when s_0 is less than 5 percent, product inhibition is not particularly strong, thus arguing that: "from a practical standpoint, . . . , cheddar cheese whey (4.9% lactose, 0.2% lactic acid) might be fermented adequately in a single stage fermentor, whereas cottage cheese whey (5.8% lactose, 0.7% lactic acid) benefits from an additional stage." Illustrate the magnitude of this benefit by repeating part (*b*) design using $s_0 = 5.8\%$, $p_0 = 0.7\%$.

(*b*) These authors also point out that addition of sugar would reduce the amount of water which it would be necessary to remove to get a fixed mass of product. How would sugar addition affect a reactor design strategy?

9.16 Staged fermentations; hydrocarbons Let us suppose that you have the batch growth curve for the hydrocarbon fermentation described so vividly by Mimura et al. in Sec. 8.8. In Prob. 8.11, you identified the probable controlling resistances of each of the fermentation phases observed. Your company has decided (in your absence) to scale up this fermentation by n tanks in series, where n = number of tanks = number of distinct cell-bubble-substrate configurational phases reported in Fig. 8.14. For each phase, write down the controlling resistance(s) and discuss quantitatively how you would scale the reactor volume, power inputs, etc., to obtain a scale factor of 5000 from laboratory to process units.

9.17 Penicillin fermentation The results shown in Fig. 9P17.1 were obtained from a *Penicillium chrysogenum* fermentation for penicillin production. The experiment was run in a well-stirred ten-liter fed-batch fermentor, aerated at 0.2 VVM (gas volumes per fermentor volume per minute). The growth and production medium contained initially (in grams per liter):

KH_2PO_4: 3.0; Na_2SO_4: 0.9; MgCl: 0.25; $MgSO_4 \cdot H_2O$: 0.05; Glucose: 10; NH_4Cl: 2

In addition, glucose was fed continuously and NH_4OH was used for pH control. Benzil penicillin has the following formula: $C_{16}H_{18}N_2O_4S$.

(*a*) Explain the profiles for cell mass, penicillin, glucose and NH_3 in this fed-batch fermentation.

(*b*) Notice that very unexpectedly, the biosynthesis of penicillin came to a rapid halt even though this organism has the capability for synthesizing five times the amount of penicillin accumulated at the point its synthesis ceased. As chief trouble shooter for Antibiotics Unlimited, Ltd., you are requested to solve the mystery of why penicillin synthesis stopped.

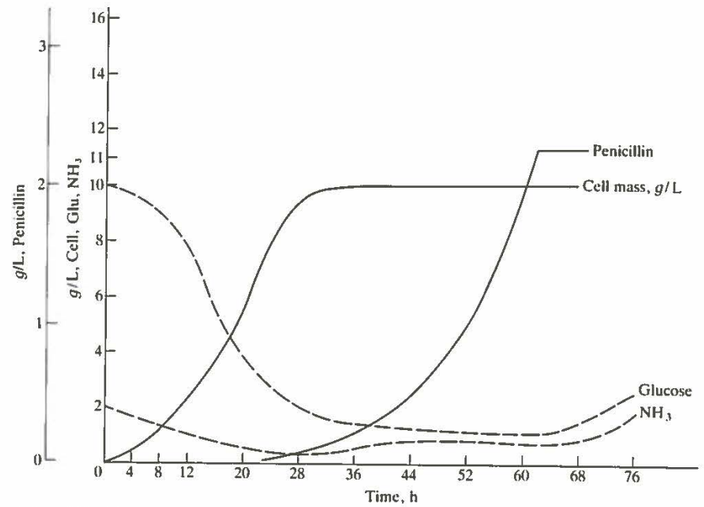

Figure 9P17.1 Time course of a batch penicillin fermentation.

9.18 Cyclic batch operations It is difficult to administer continuously low, controlled liquid feed rates, whereas the comparable periodic stepwise addition of substrate feed removal of fermentor fluid is easier. Suppose microorganism growth is limited by a single substrate in a Monod fashion, and that cell growth is proportional to the time rate of substrate change. At time = 0, the fermentation is begun (negligible lag). At time t, a volume V_1 is removed from the fermentor of liquid volume V, it is replaced by an identical volume of fresh feed at concentrationss_f.

(*a*) Show that at time t (before volume removal), the substrate concentration s is given by

$$\left[1+\frac{K_s}{(x_0/Y)+s_0}\right]\ln\left[1+\frac{1-s/s_0}{x_0/(Ys_0)}\right]-\frac{K_s}{(x_0/Y)+s_0}\ln\left[\frac{s}{s_0}\right]=\mu_m t$$

(*b*) At time t, the volume V_1 is removed, now feed is added, and the process repeats. Evaluate s_0, x_0 in terms of s, x_t from this volume operation.

(*c*) Then establish that the substrate utilization efficiency

$$\beta \equiv (s_f - s_1)/s_f = 1 - \frac{\phi}{1-\phi}\left(e^{\mu(Vt/V_1)s_f\phi/K_s}[1-\phi]^{s_f/K_s}-1\right)^{-1}$$

where $\phi = V_1/V$.

(*d*) As $\phi \to 0$, show that this efficiency becomes identical with the continuous culture result

$$\beta = 1 - \frac{K_s}{s_f[\mu_m(V/V_1)t - 1]}$$

where Vt/V_1 is the reciprocal dilution rate.

(*e*) Show graphically that at $s_f/K_s = 1.0$, finite values of ϕ (discrete operation) give higher β values than the continuous reactor if $(\mu_m Vt/V_1) > 4$.

9.19 Fluidized bed tissue culture It is proposed to produce tissue culture biomass continuously on nonporous beads in a fluidized bed (Fig. 9P19.1). The proposed fluidizing nutrient liquid moves

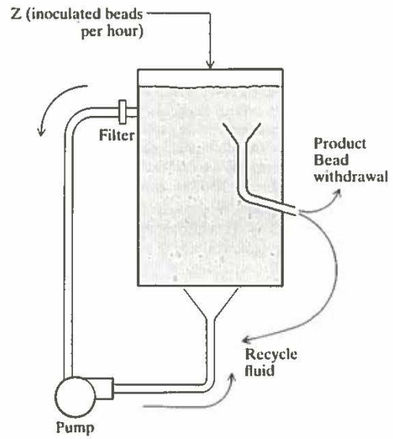

Figure 9P19.1 Fluidized bed microcarrier bioreactor for tissue culture.

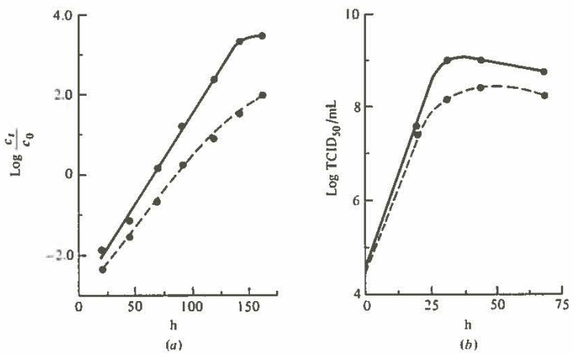

Figure 9P19.2 Production of cell number and viral titer vs. time. (*a*) c_t (cells attached at time t)/c_0 (cells inoculated at time $t = 0$) vs. time (———75% O_2 saturation; -------5% O_2 saturation). (*b*) $TCID_{50}$ (≡ virus dose yielding 50 percent tissue culture infection dose per milliliter) vs. time (———13×10^5 cells/milliliter initial cell concentration; -------3.5×10^5 cells/milliliter initial cell concentration). *[Reprinted from A. L. van Wezel, Microcarrier Cultures of Animal Cells, in "Tissue Culture: Methods and Applications," p. 372, Academic Press, Inc., New York, 1973.]*

upward, passing out of the reactor through a screen to retain beads, and then through a pump. The choice of beads with $(\rho_{bead} - \rho_{H_2O})$ small allows operation at a low fluidizing volume rate and use of a pump which does not damage the macromolecules in the nutrient medium. For liquid fluidized reactors, assume that the liquid moves in plug flow while the beads are perfectly mixed and distributed throughout the reactor. Beads passing over the exit funnel settle into it and are continuously removed; the exit fluid being returned by a subsequent filter.

(*a*) Assuming the data of Fig. 9P19.2 to typify cell growth kinetics on each bead with $\mu = 0$ for $t > 160$ h [due to achievement of a complete monolayer (contact inhibition)], develop a steady state expression for the exit biomass concentration per bead in terms of the fluid bed void volume ε, the feed rate of inoculated beads (Z per hour), the reactor volume V_R and the appropriate growth rate law. The entering beads have c_0 cells/bead.

(*b*) Repeat (*a*) for the situation where the cells per entering bead have a distribution given by

$$\text{Prob of } (y) \text{ cells/bead} = Ae^{-(y-y_m)^2}$$

where A = normalizing factor

(*c*) How would you design a "continuous," sterile transport system for adding new beads and for recovering the exit liquid volume as assumed?

9.20 Tissue culture support inhibition It has been observed that high concentrations (cm^2/cm^3) of solid supports for tissue culture may be deleterious to growth because of adsorption on these surfaces of growth enhancing factors and serum protein from the support medium, adsorption rendering these factors inaccessible (C. B. Horng and W. McLimens, *Biotech. Bioeng.*, **17**: 713, 1975). Consider a well-stirred microcarrier tissue culture reactor employing nearly neutrally bouyant beads as the cell supports.

(*a*) Assuming that the cells grow logarithmically (all nutrients present in excess) until the available surface area is covered (see Figure 9P19.2), develop an expression for the total biomass in the system versus time.

(*b*) Now assume that the growth rate also depends in a Monod function fashion upon the concentration of a growth-enhancing factor, S_g, which is not consumed. Derive the analogous result for biomass versus time when the growth enhancing factor S_g reversibly adsorbs on the solid in an inactive form following a linear partition law:

$$s_g(l) = Ks_g(\text{adsorbed})$$

(*c*) For a batch system, assuming that growth exhaustion results from lack of further bead surface, show that for a finite culture time (say 6 days) there is an optimal initial loading of beads into the tissue culture inoculum. Develop an expression for this value.

(*d*) Since the beads act in some sense as an inhibitor, it would seem logical to consider several stirred tanks in series rather than one tank. What problems would face the experimenter searching to set up such a system?

9.21 Penicillin-V deacylation: multiple reactions Enzymatic deacylation of penicillin-V to produce the desired 6-aminopenicillanic acid (Chap. 12) for production of semisynthetic penicillins involves a reaction network represented below:

$$A^-(\text{pen.-V}) \xrightarrow{r_1} P^-(\text{6-APA}) + \text{QOH(phenoxyacetic acid)}$$

$$A^- \xrightarrow{r_2} R^{2-} \text{ (inactive product)} + H^+$$

$$P^- \xrightarrow{r_3} HS^- \text{ (inactive ring cleavage product)}$$

$$QOH \underset{2}{\overset{K_1}{\rightleftharpoons}} QO^- + H^+$$

$$P^- + H^+ \overset{K_2}{\rightleftharpoons} HP \text{ (stable penicillin-V)}$$

An optimal pH exists because protonation of P^- gives stable HP, but too acidic conditions inhibit the reaction rate r_1.

(*a*) Assuming r_1 given by Michaelis-Menten kinetics with noncompetitive H^+ inhibition, and r_2 and r_3 given by simple first-order irreversible forms, write down the equations governing these three rates and two (assumed) equilibria.

(*b*) For a steady-state CSTR system with perfect enzyme recycle, derive an expression giving the feed level of A^- which maximizes fractional conversion to P^-.

(*c*) For very high conversion of an expensive starting material, use of several CSTR's in series appears appropriate. Discuss tactically the advantages and disadvantages attending such a series arrangement. Assume r_2, r_3 are about 2-5 percent of the rate r_1 *in the first tank*.

(L. G. Karlsen and J. Villadsen, "Optimization of a Reactor Assembly for the Production of 6-APA from Penicillin-V," *Biotech. Bioeng.*, **26**: 1485, 1984).

9.22 Hollow fiber reactor productivity Different reactor types can yield profoundly different microbial densities and productivities. For example, β-lactamase specific production activity is less in hollow fiber systems than in continuous culture by a factor of five:

Reactor	Productivity (units E/cell-h)
Shaker flask	1×10^{-10}
Hollow fiber	2×10^{-11}

(*a*) If the biomass level in the hollow fiber reactor is (typically) 1000 times greater than in suspension culture, calculate the productivity of each reactor in units/(reactor volume − h), assuming a shake flask biomass level of $x = 10^9$ cells/mL.

(*b*) Cell lysis in the hollow fiber, as well as protein export, accounted for some increases in β-lactamase release. Describe a program by which you would determine cell lysis kinetics, which could then be used to describe β-lactamase production by excretion and lytic release. (See D. S. Inloes et al, "Hollow Fiber Membrane Bioreactors Using Immobilized *E. coli* for Protein Synthesis," *Biotech. Bioeng.*, **25**: 2653, 1983).

9.23 Batch production of non-growth associated product Batch production typically involves biomass production and product formation, with substrate being consumed by each process. Suppose that batch growth is modelled by the logistic equation, where x_{max} is set by the initial value of a second substrate s_2, (not used in product formation). Then for a nongrowth associated product (e.g. L-glutamate from *Micrococcus glutamicus*), we may write;

$$dx/dt = \mu x(1 - x/x_{max})$$
$$dp/dt = nx$$
$$ds/dt = -(dp/dt)/Y_p - (dx/dt)/Y_x$$

(*a*) Integrate each equation to obtain $x(t)$, $p(t)$, $s(t)$.

(*b*) Discuss your strategy in setting x_{max} if your objective is (i) to maximize $p(\theta)$ or (ii) to maximize $p(\theta)/\Delta s(\theta)$, where θ is a fixed, end-of-fermentation time.

(*c*) Under what circumstances would you choose objective (i) or (ii) in part (*b*)?

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CHAPTER

TEN

INSTRUMENTATION AND CONTROL

We have seen repeatedly that the activity and useful lifetime of an enzyme catalyst or cell population depends directly on the catalyst environment. Accordingly, in order to develop and optimize biological reactors and in order to operate them most efficiently, it is critical that the state of the catalyst environment be monitored and controlled and that the response of the catalyst to the environment be determined. Achieving these goals requires three different functions: measurement, analysis of measurement data, and control. In this chapter we will examine currently available reactor instrumentation and its application. After a brief look at the rapidly changing technologies for data acquisition and computing, we shall summarize some of the strategies for data analysis and process control. Although this chapter's presentation emphasizes bioreactor instrumentation and control, the principles described also apply to downstream processing and to feedstock preparation.

10.1 PHYSICAL AND CHEMICAL SENSORS FOR THE MEDIUM AND GASES

Figure 10.1 shows a recently prepared schematic summary of biochemical reactor instrumentation. A striking feature of this illustration is the predominance of measurements of medium and gas chemical and physical properties and the shortage of measurements of cell properties. Consequently, one of the major

goals, if not requirements, of bioreactor data analysis is estimation of cell properties based on the available physiochemical measurements of the gas streams and the medium. In this section we will concentrate on instrumentation for on-line physical and chemical monitoring of bioreactors.

10.1.1 Sensors of the Physical Environment

The major physical process parameters that influence cellular function and process economics and which can be monitored continuously are temperature, pressure, agitator shaft power, impeller speed, broth viscosity, gas and liquid flow rates, foaming, and reactor contents volume or mass. In small laboratory reactors, only temperatures and air-feed flow rates are commonly measured. Pressure measurement and regulation is common on larger fermentors.

The most widely used temperature sensor is the thermistor, a semiconductor device which exhibits changing resistance as a function of temperature. Although the temperature-resistance relationship is nonlinear, this is not a serious difficulty over the narrow temperature range of interest for most fermentations (25–45°C). Other possible temperature sensors are the platinum resistance sensor, thermometer bulbs (Hg in stainless steel), and thermocouples.

Pressure monitoring is important during sterilization, and maintaining a positive reactor head pressure (around 1.2 atm absolute) can aid in preserving asepsis. Pressure also influences gas solubility. In fermentation reactors, diaphragm gauges are usually used to monitor pressure. These produce a pneumatic signal which may be transduced if necessary to an electrical signal.

Several different types of measurements can be made to monitor power input in mechanically agitated vessels. A Hall effect wattmeter measures at the drive motor armature the total energy consumed by the agitator. A torsion dynamometer may also be used to measure shaft power input. A disadvantage of both of these measurement methods is inclusion of frictional losses in shaft bearings and seals. For example, in a study of mixing in a 270-liter fermentor with 200 liters working volume, it was found that 30% of the energy used by the motor was lost between the motor and the internal impeller shaft. This loss factor was also observed to be an increasing function of agitator speed. Direct measurement of impeller power input to the reactor fluid may be achieved using balanced strain gauges mounted on the impeller shaft inside the reactor.

On-line devices for measuring broth viscosity and other rheological properties are not well developed. One possible strategy is measurement of power consumption at several different impeller speeds. Also, a dynamic method has been proposed in which shaft power input is monitored during and after a brief (less than 30 s) shutoff in agitator drive power. As sketched in Fig. 10.1, Newtonian and non-Newtonian broths have been observed to respond differently during such a brief agitation transient.

Several different instruments are available for measuring flow rates of gases (air feed, exhaust gas). The simplest, a variable area flowmeter such as a rotameter, provides visual readout or may be fitted with a transducer to give an

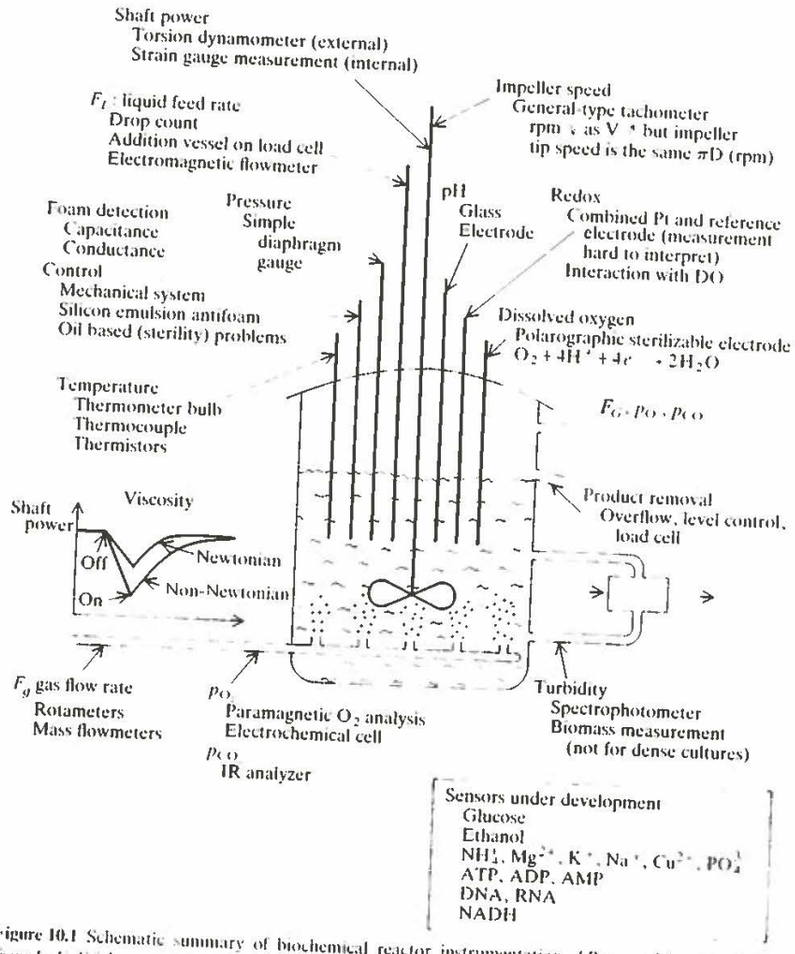

Figure 10.1 Schematic summary of biochemical reactor instrumentation *[Reprinted by permission from T. E. Erickson and G. Stephanopoulos, "Biological Reactors," Chap. 13 in "Chemical Reaction and Reactor Engineering," J. J. Carberry and A. Varma (eds.), Marcel Dekker, Inc., New York, 1985.]*

electrical output. Thermal mass flowmeters are increasingly popular, especially for lab and pilot-scale reactors. In these devices, gas flows through a heated section of tubing, and the temperature difference across this heated section is directly related to mass flow rate. These instruments have accuracies on the order of 1% of full-scale and are most useful for flow rates less than 500 L/min. Also available are laminar flow measurement devices which determine flow based on differential pressure drop across a matrix device which divides the total flow into

multiple parallel capillary flows. Gas flow rate measurements are important since these quantities are used frequently in material balancing calculations (Sec. 10.5).

Liquid flow rates can be monitored with electromagnetic flowmeters, but these are not used widely due to their cost. Occasionally, especially in laboratory scale studies, one relies on a metering or other well-calibrated pump to provide the desired liquid flow rate. Alternatively, liquid can be added to the reactor in discrete doses of well-defined volume or mass. Long-term monitoring of net flow into the vessel may be achieved by continuous weighing of the reactor and its liquid contents using a strain gauge (vessels > 250 L) or scale (smaller vessels). Alternatively, a liquid level sensor based on a capacitance probe may be used to monitor reactor liquid content. Such capacitance probes or a conductance probe may also be used to detect buildup of foam on the top surface of the reactor contents. In some situations an external loop of circulating broth is used for measurements (see below), to effect product removal and cell recycle, or for heat and or gas exchange as discussed in Chaps. 9 and 14. Here the presence of suspended particulates and changing broth rheology severely complicate liquid flow rate measurement.

10.1.2 Medium Chemical Sensors

Electrodes which can be repeatedly steam-sterilized in place are now available for pH, redox potential (E_h) and dissolved oxygen and CO_2 partial pressures. The most widely used and reliable probe among these is the pH electrode, which is generally a single unit glass-reference electrode design. A schematic diagram of a pH electrode designed for autoclave sterilization is shown in Fig. 10.2. Electrodes for *in situ* sterilization must include a housing to provide pressure balance during sterilization or pressurized bioreactor operation. Measurement of medium redox potential is possible using a combined platinum and reference electrode. Combined pH-redox probes are available. While the influence of pH on biochemical kinetics is clearly established and the physical significance of a pH measurement is straightforward, interpretation of redox potential measurements and understanding the relationship between redox potential and cell activity can be difficult. One promising application of redox measurements is in monitoring low contents of dissolved oxygen (< 1 ppm) in anaerobic processes (−450 mV < E_h < −150 mV) where product formation may be quite sensitive to E_h.

The various types of dissolved oxygen probes now available are of galvanic (potentiometric) or polarographic (amperometric or Clark) types. These electrodes measure the partial pressure (or activity) of the dissolved oxygen and not the dissolved oxygen concentration. In both designs, an oxygen-permeable membrane usually separates the electrode internals from the medium fluid (Fig. 10.3). Also, both designs share the common feature of reduction of oxygen at the cathode surface.

Cathode

$$\tfrac{1}{2}O_2 + H_2O + 2e \xrightarrow{Pt} 2OH \qquad (10.1)$$

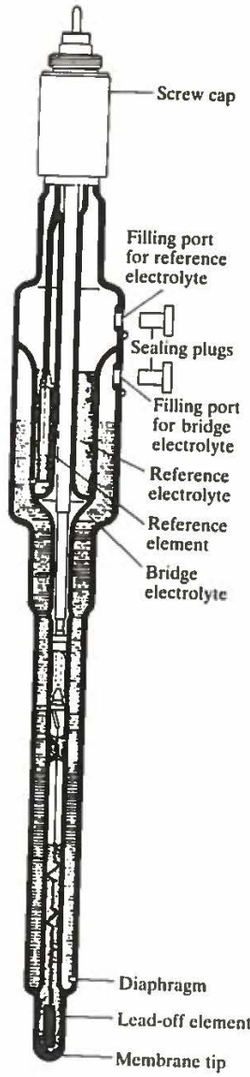

Figure 10.2 Schematic diagram of a combination pH electrode designed for autoclave steam sterilization. *(Ingold type 465. Illustration courtesy of Ingold Electrodes Inc.)*

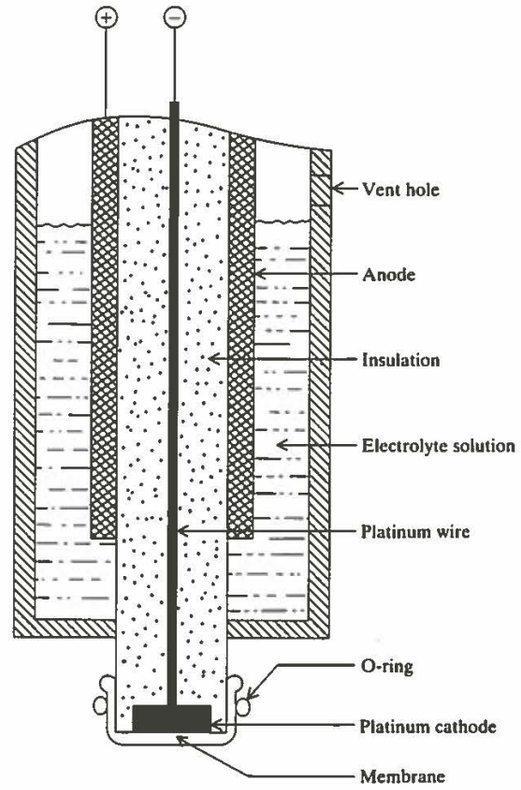

Figure 10.3 Schematic diagram of major components of an electrochemical probe for measurement of dissolved oxygen partial pressure. *(Reprinted by permission from N. S. Wang and G. Stephanopoulos, "Computer Applications to Fermentation Processes," CRC Critical Reviews in Biotechnology, vol. 2, p. 1. © CRC Press, Inc., 1974. Used by permission of CRC Press, Inc.)*

The reaction at the anode in a galvanic electrode

Anode (galvanic)

$$Pb \longrightarrow Pb^{2+} + 2e^- \tag{10.2}$$

completes the cell from which a small amount of current is drawn to provide a voltage measurement which in turn is correlated to the oxygen flux reaching the cathode surface. In a polarographic type of oxygen electrode, a constant voltage is applied across the cathode [Eq. (10.1)] and anode

Anode (polarographic)

$$Ag + Cl^- \longrightarrow AgCl + e^- \tag{10.3}$$

and the resulting current, which depends on the oxygen flux to the cathode, is measured. Drift caused by accumulation of hydroxyl or metal ions or chloride

depletion is a common drawback of both electrode types. External fouling of the membrane surface may also contribute to drift.

In steady state, the oxygen flux at the cathode depends upon a series of transport steps in which oxygen moves from the bulk liquid to the outer membrane surface, diffuses through the membrane, and finally diffuses through the electrolyte solution to the cathode surface where reaction occurs effectively instantaneously. To the extent that the first step limits the overall transport rate, and thus the oxygen flux to the cathode, the electrode output will depend on fluid properties (e.g., viscosity) and local hydrodynamic conditions near the electrode. For this reason it has been recommended, for example, that the fluid velocity at the tip of a polarographic electrode should be at least 0.55 m/s. Sensitivity of the electrode output to external boundary layer transport can also be reduced by using a less permeable membrane. (Why?) This approach has the disadvantage of introducing additional time delay in the instantaneous electrode response to transients in dissolved oxygen partial pressures. The characteristic response time of membrane-covered dissolved oxygen sensors is quite long (10–100 s). However, as shown in the following example, transient probe measurements may still be applied to characterize mass-transfer properties of bioreactors provided the influence of diffusion through the probe membrane is included in the analysis.

Example 10.1: Electrochemical determination of $k_l a$ An oxygen electrode is inserted into a steadily aerated batch or continuous-flow reactor. After a steady electrode response has been achieved, the oxygen-carrying flow is suddenly replaced by an equivalent nitrogen flow, which then strips the oxygen from solution in a transient manner. Under these circumstances, the voltage response of the probe is given by [11]

$$\frac{E(t)}{E_0} = \left[\tau^{1/2}\exp(-\beta t) - \sin\tau^{1/2} - 2\sum_{n=1}^{\infty}(-1)^n \exp(-n^2\pi^2\mathcal{D}_{O_2} t/L^2)\right] \Big/ \left[1 - n^2\pi^2\tau\right] \qquad (10E1.1)$$

where $\tau = \beta L^2/\mathcal{D}_{O_2}$

$E(t)$ probe voltage at time t
E_0 probe voltage at time 0
F_g gas flow rate
V liquid volume in tank
M Henry's law constant
L thickness of probe membrane
$\mathcal{D}_{O_2}$ oxygen diffusivity in probe membrane
P_m permeability membrane thickness, and

$$\frac{1}{\beta} \text{ time constant of system (reactor)} \quad \frac{1}{k_l a} + \frac{VRI}{F_g M} \qquad (10E1.2)$$

If the system has a large time constant (small $k_l a$), the value of β is determined from electrode-voltage values for times much greater than the electrode response itself, in which case the series contribution above is negligible. For larger values of $k_l a$ (50–500 h^{-1}), Wernan and Wilke [11] suggest using the slope of the electrode response at the inflection point (where the second derivative of E with respect to t vanishes). This method was found to give results as accurate as computer-aided curve fitting using the entire voltage-time response. For various electrode relaxation times, the value of the inverse relaxation time is read from a graph of this parameter vs. the slope of E vs. t at the inflection point.

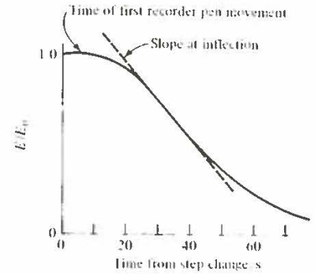

Figure 10E1.1 The inflection-point slope is determined graphically from the electrode response following a switch from sparging of an oxygen-carrying gas to nitrogen sparging. (*Reprinted from H. C. Wernan and C. R. Wilke, "New Method for Evaluation of Dissolved Oxygen Response for $k_l a$ Determination," Biotech. Bioeng., vol. 15, p. 571, 1973.*)

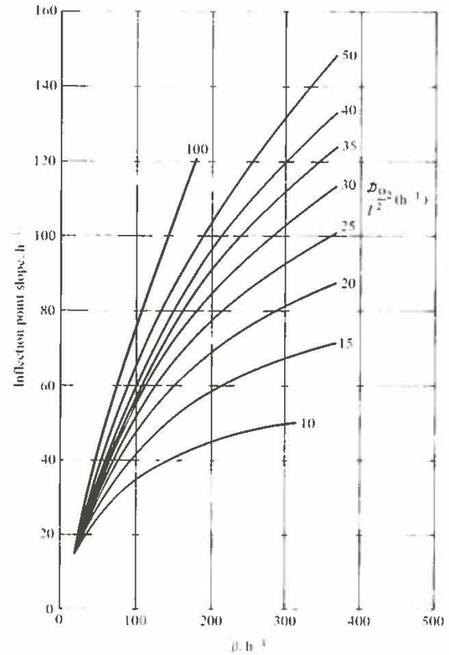

Figure 10E1.2 From this graph, the value β may be determined from the inflection-point slope and membrane-transport parameters. $k_l a$ then follows from Eq. (10E1.2). (*Reprinted from H. C. Wernan and C. R. Wilke, "New Method for Evaluation of Dissolved Oxygen Response for $k_l a$ Determination," Biotech. Bioeng., vol. 15, p. 571, 1973.*)

Evaluation of the desired value, k_la, then proceeds in three steps

1. From the E vs. t electrode response curve (Fig. 10F1.1) evaluate the slope at the inflection point (steepest point).
2. From the generalized display of Eq. (10F1.2) in Fig. 10F1.2, obtain β, given the slope and the electrode time constant.
3. Evaluate k_la from the definition of the system time constant ($1/\beta$) above.

It is evident that the usefulness of this technique depends on the accuracy with which the electrode response is known, particularly for large k_la values.

The presence of high solute concentrations may alter oxygen solubility (Table 8.1), the oxygen diffusion coefficient and the electrode itself (thus the electrode response). A complete dissection of these influences clearly requires calibration by independent means.

Steam-sterilizable electrochemical probes for dissolved CO_2 partial pressure have been introduced relatively recently. The CO_2 probe produced by Ingold, for example, determines p_{CO_2} by measuring the pH of a standard bicarbonate solution which is separated from the process fluid by a gas-permeable membrane. Calibration is accomplished by measuring pH after substitution of a reference buffer solution for the bicarbonate solution.

Another class of methods for on-line assay of volatile medium components and dissolved gases is based upon immersion of a length of tubing, permeable to the component(s) of interest, in the fluid to be analyzed. Continuous flow of a carrier gas through the tubing sweeps the compounds which penetrate the tubing to a gas analysis device (see next section), where the measurement is conducted. This approach suffers from substantial measurement delays (2–10 min) and, therefore, is not optimal for monitoring rapid transients in concentrations.

Several *biosensors* have been developed for assay of specific components in the liquid phase. These are based on coupling the action of immobilized enzymes or cells with an analytical device which detects a particular product of the bio-catalyzed reaction. Examples of combining enzyme-catalyzed reactions with electrochemical detectors were discussed earlier in Sec. 4.3.3. Also studied extensively are enzyme thermisters, in which the heat released by the enzyme-catalyzed reaction is detected by a nearby calorimeter. Table 10.1 summarizes some of the compounds which have been assayed by this method and the corresponding enzymes employed. Other possibilities for biosensor development using immobilized enzymes include enzyme transistors in which reaction products (for example, hydrogen) cause changes in the electronic properties of solid-state devices (for example, silicon chips with an SiO_2-layer covered with a Pd film).

The spectrum of biosensor designs and configurations can be enlarged by considering a broader class of biocatalyzed reactions, including multistep or coupled reactions, to generate the detected component. Immobilized cells provide a convenient means in many cases for transforming the component to be assayed into a suitable detectable compound. Immobilized whole-cell respiratory activity (assay by oxygen electrode) and production or consumption of electroactive metabolites by whole cells (assayed by fuel cell electrode or by pH or CO_2 electrodes) have been used as the bases for design and application of biosensors

Table 10.1 Examples of analytical applications of enzyme thermistors
(Adapted from B. Danielsson and K. Mosbach, "The Prospects for Enzyme-Coupled Probes in Fermentation," p. 137, in "Computer Applications in Fermentation Technology," Society of Chemical Industry, London, 1982.)

Substance analyzed	Immobilized enzyme	Concentration range or detection limit, mmole l
Ascorbic acid	Ascorbic acid oxidase	0.05–0.6
Albumin (antigen)	Immobilized antibodies + enzyme-linked antigen	10^{-10}
ATP	Apyrase or hexokinase	1–8
Cellobiose	β-glucosidase + glucose oxidase catalase	0.05–5
Cephalosporin	Cephalosporinase	0.005–10
Cholesterol	Cholesterol oxidase	0.03–0.15
Cholesterol esters	Cholesterol esterase + cholesterol oxidase	0.03–0.15
Creatinine	Creatinine iminohydrolase	0.01–10
Ethanol	Alcohol oxidase	0.01–1
Galactose	Galactose oxidase	0.01–1
Gentamicin (antigen)	Immobilized antibodies + enzyme-linked antigen	0.1 μg ml
Glucose	Glucose oxidase/catalase	0.002–0.8
Heavy metal ions (e.g., Pb^{2+})	Urease	10^{-6}
Insecticides (e.g., parathion)	Acetylcholinesterase	5×10^{-5}
Insulin (antigen)	Immobilized antigen + enzyme-linked antigen	0.1–1.0 unit ml
Lactate	Lactate 2-monooxygenase	0.01–1
Lactose	Lactase and glucose oxidase/catalase	0.05–10
Oxalic acid	Oxalate oxidase	0.005–0.5
Penicillin G	Penicillinase	0.01–500
Phenol (substrate)	Tyrosinase	0.01–1
Sucrose	Invertase	0.05–100
Triglycerides	Lipase, lipoprotein	0.1–5
Urea	Urease	0.01–500
Uric acid	Uricase	0.5–4

containing immobilized whole cells. Table 10.2 summarizes the properties of a number of whole-cell sensors which are described in greater detail in Ref. 12.

An interesting and promising alternative strategy for formulating specific "affinity sensors" for individual metabolites has been described and developed by J. S. Schultz and coworkers [13]. The requirements for this method are a specific binding agent for the component to be assayed, availability of a suitably labeled component which competes for the same specific binding agent, and a means of separating binding agent from the solution to be assayed. For example, a fiber optic fluorescence probe has been constructed for glucose analysis by immobilizing concanavalin A (Con-A), a protein from jack bean which selectively binds sugars, on the internal surfaces of a measurement chamber. The chamber, separated from the assayed solution by a dialysis membrane permeable to glucose,

Table 10.2 Summary of microbial sensors for process fluid analysis

Component assayed	Organism employed	Immobilization method	Measurement principle	Detection device
Acetic acid, ethanol	*Trichosporon brassicae*	Attachment to porous acetylcellulose	Simultaneous O_2 consumption	O_2 electrode
Ammonia	*Nitrosomonas* and *Nitrobacter* species	Rentention by gas-permeable membrane	Simultaneous O_2 consumption	O_2 electrode
Cephalosporin	*Citrobacter freundii*	Collagen membrane entrapment	Liberation of H^+	pH electrode
Formic acid	*Clostridium butyricum*	Agar entrapment on acetylcellulose filter	Production of H_2	Fuel cell electrode
Glucose	*Pseudomonas fluorescens*	Collagen membrane entrapment	Simultaneous O_2 consumption	O_2 electrode
Glutamic acid	*Escherichia coli*	Rentention by cellophane membrane	CO_2 production	CO_2 electrode

also contains dextran labeled with the fluorochrome fluoroscein isothiocyanate (FITC). The membrane used is impermeable to the FITC-dextran which competes with glucose for binding to Con-A:

$$\text{Glucose} + \text{Con-A} \rightleftharpoons \text{Con-A-glucose}$$
$$\text{FITC-Dextran} + \text{Con-A} \rightleftharpoons \text{Con-A-FITC-dextran}$$

Thus, the amount of unbound FITC-dextran, and hence the measured fluorescence emission intensity, is a function of the solution glucose concentration. Interference by other solutes which also bind to the specific binding agent (maltose, sucrose, and fructose to Con-A, for example) pose potential problems for this approach, but the concept involved is intriguing, quite general, and should enjoy further development.

A further concern in use of any sensor employing enzymes, cells, or other biochemicals is deactivation of the sensor during reactor sterilization. Mechanical designs which allow aseptic removal and insertion of the sensor in the reactor interior have now been developed to address this potential problem. Also, as in all sensors which depend on transport of the monitored component through a membrane, membrane fouling by cells or medium components and external mass transport resistance can cause drift or shifts in calibration of the sensor.

10.1.3 Gas Analysis

The concentration of CO_2 in the exhaust gas from a cell reactor is indicative of respiratory or fermentative activity of the organisms and hence is one of the most useful and widely applied measurements in monitoring and controlling a cell bioreactor. CO_2 content in bioreactor gas streams is most commonly monitored using an infrared spectrophotometer. The gas sample stream must be dessicated carefully before entering the instrument to avoid damage to the sample cell windows. Gas stream CO_2 concentration may also be measured using thermal conductivity, gas chromatography, or mass spectrometry.

Gas stream oxygen partial pressure is usually measured using a paramagnetic analyzer. Here too, elimination of water vapor in the sample stream is essential to minimize drift, and the sample stream flow rate must be controlled carefully for consistent measurements. Paramagnetic analyzers are also quite sensitive to small changes in total atmospheric pressure, requiring simultaneous monitoring of barometric pressure for compensation in oxygen analysis. Drift in readings which necessitate on-line recalibration is a frequent occurrence with paramagnetic analyzers when applied to fermentations.

Gas chromatography (GC) can be applied to analyze several components of the exhaust gas stream including O_2, CO_2, CH_4 (e.g., in anaerobic methane generation), and H_2 (from *Hydrogenomonas* cultures, for example). Also, by determining the gas phase partial pressure of volatile components such as ethanol, acetaldehyde, and carboxylic acids, GC measurements provide useful information on the status of the fermentation and on the liquid phase concentrations of these compounds. The requirement of intermittent injection of samples (ca. 15 min apart) limits the utility of GC measurements for monitoring process transients.

Mass spectrometry (MS) is enjoying increasing popularity for monitoring gas stream composition. Lower-priced instruments are making MS more accessible for research applications, and reliable, robust process instruments have made mass spectrometry more practical for industrial application. MS instruments offer rapid response times (< 1 min), high sensitivity (around 10^{-5} M detection limit), capability to analyze several components essentially simultaneously, linear response over a broad concentration range, and negligible calibration drift. Because of the expense of MS instruments, it is often desirable to interface the analyzer to several bioreactors and use a computer-controlled switching manifold to cycle sample streams from different reactors into the MS (Fig. 10.4). As indicated in this schematic diagram (only three fermentors are shown, but a single mass spec can support up to 30), the same computer may also be used for process control.

Often, standard values (20.91 % O_2, 0.03 % CO_2) are assumed for the feed air composition, but it is sometimes more reliable to measure feed gas composition directly by including a feed gas sample stream in the manifolding arrangement as indicated in Fig. 10.4. Of course, the merits of sharing analyzer instrumentation by use of such multiplexing and manifold arrangements are not limited to cases in which mass spectrometry analyzers are applied.

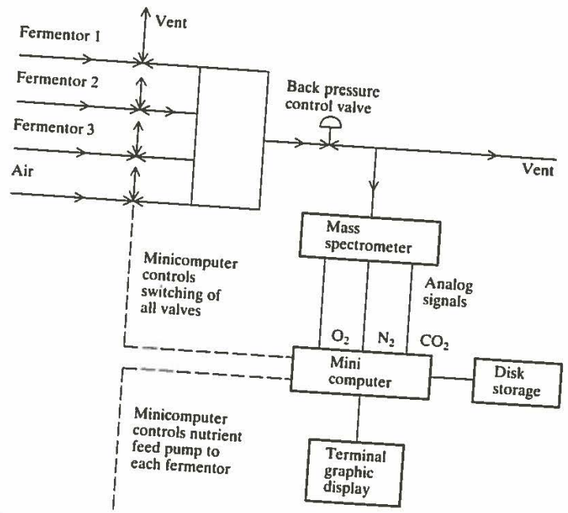

Figure 10.4 Schematic illustration of a computer-controlled sample selection system for time-shared use of a mass spectrometer. *(Reprinted by permission from R. C. Buckland and H. Fastert, "Analysis of Fermentation Exhaust Gas Using a Mass Spectrometer," p. 119, in "Computer Applications in Fermentation Technology," Society of Chemical Industry, London, 1982.)*

10.2 ON-LINE SENSORS FOR CELL PROPERTIES

Unfortunately, there are few instruments for continuous monitoring of cell properties in a bioreactor. The most basic measurement needed is total biomass content or concentration or, better still, active biomass concentration. Although a number of possible methods exist, no approach has yet been invented which provides such data reliably, consistently, and for a broad class of organisms and media.

Optical methods based upon light absorbance (spectrophotometry) or scattering (nephelometry, reflectance measurement) have been investigated widely. A sample stream from the reactor may be circulated through a spectrophotometer. A potential difficulty here is the nonlinearity between optical density and biomass concentration above O.D. = 0.5 or 0.5 g biomass/L. Consequently, sample stream dilution or a shorter light path may be used for measurement of dense cultures. Figure 10.5 shows a flow-through cuvette design developed by Lim and colleagues which has proved very convenient in a number of laboratories. Alternatively, probes which can be inserted into the process fluid for optical cell density measurements have been developed.

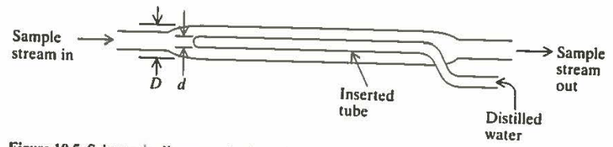

Figure 10.5 Schematic diagram of a flow-through cuvette with internal effective dilution by means of an inserted tube containing distilled water. With $d = 8$ mm, $D = 12$ mm, this device provides a linear OD_{600}-cell density relationship for cell densities exceeding 1.8 g/L. *(Adapted from C. Lee and H. Lim, "New Device for Continuously Monitoring the Optical Density of Concentrated Microbial Cultures," Biotech. Bioeng., vol. 22, p. 636, 1980.)*

The only continuous monitoring strategy so far developed that provides information on the biochemical or metabolic state of the cell population is in situ fluorometry. Ultraviolet light (366 nm wavelength) is directed into the culture. Excited by this incident UV radiation, reduced pyridine nucleotides (NADH and NADPH) fluoresce with a maximum intensity at approximately 460 nm. The fluorescence emitted from the culture is measured with a suitable detector such as a photodiode or photomultiplier. Originally, these measurements were made through quartz windows installed in the walls of laboratory fermentors. The advent of fluorescence probes which can be used in standard electrode ports in fermentation vessels should increase investigations and application of culture fluorescence measurements (Fig. 10.6).

Culture fluorescence intensity depends on cell density, average cell metabolic state and fluorescence emissions, and light absorption by the medium. Experiments in particulate-free media have shown that culture fluorescence measurements provide useful information on biomass concentration, oxygen transfer and

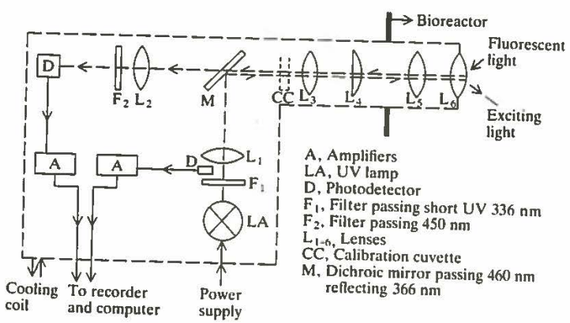

Figure 10.6 Internal components in one design for an *in situ* fluorescence probe. *(Reprinted by permission from W. Beyeler, A. Einsele and A. Fiechter, "On-Line Measurements of Culture Fluorescence: Method and Application," European J. Appl. Microbial. Biotechnol., vol. 13, p. 10, 1981.)*

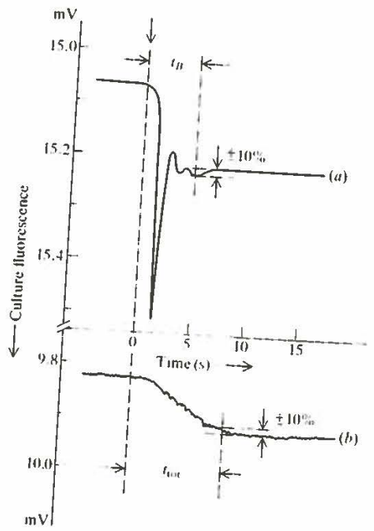

Figure 10.7 Transients in fluorescence measured (*a*) after pulse addition of quinine to 0.5 *M* H_2SO_4 solution, which characterizes bulk liquid mixing, and (*b*) after glucose addition to a yeast culture, which characterizes liquid mixing plus cellular glucose uptake. (*Reprinted by permission from A. Einsele, D. L. Ristroph and A. E. Humphrey, "Mixing Times and Glucose Uptake Measured with a Fluorometer," Biotech. Bioeng., vol. 20, p. 1487, 1978.*)

reactor mixing times, substrate exhaustion, and metabolic transients. For example, Einsele and coworkers compared the dynamics for liquid mixing in a 40-liter working volume fermentor agitated mechanically at 200 rpm with the dynamics of mixing plus glucose uptake by yeast cells. For the first measurement, fluorescence of quinine pulsed into 0.05 *M* H_2SO_4 in the reactor was monitored (this solute has approximately the same fluorescent properties as NADH). The results, shown in part *a* of Fig. 10.7, exhibit oscillations representative of a periodic circulation pattern in the vessel and provide clear evidence of significant dynamic delays in achieving new steady-state conditions in the reactor. Part *b* of Fig. 10.7 is the reduced pyridine nucleotide fluorescence from a yeast culture following a pulse of glucose added to the reactor at time zero. Here the response time is longer, indicating significant dynamic delay in glucose uptake by the cells. The implications of these features in the context of scale-up were mentioned earlier in Section 9.3.4.

Another illustration of an alternative application of culture fluorescence is monitoring of cellular metabolic state to control the fermentation. In an experimental study of *Candida utilis* grown on ethanol in a fed-batch process, culture fluorescence was used to estimate the time of substrate ethanol exhaustion and to control pulse feeding of ethanol into the process. Shown in Fig. 10.8 are some cycles of culture fluorescence, respiratory quotient, and ethanol concentration

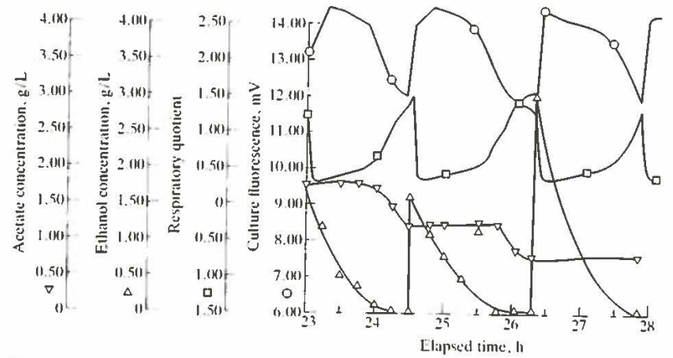

Figure 10.8 Relationship among culture fluorescence (○, mV), ethanol concentration (△, g/L), respiratory quotient (□) and acetate concentration (▽, g/L) in a culture of *C. utilis* fed with ethanol pulses. (*Reprinted by permission from C. M. Watteeux, B. B. Armiger, D. L. Ristroph, and A. E. Humphrey, "Production of Single Cell Protein from Ethanol by a Fed-Batch Process," Biotech. Bioeng., vol. 21, p. 1221, 1979.*)

during this fed-batch operation. The circles are used to identify the culture fluorescence curve the measurements are obtained continuously. In the portion of the experiment shown here, ethanol pulses of 1.85 g/L were added at 23 and 24.5 h into the batch, while the pulse at 26.15 h added 3 g ethanol per liter of culture. Reduction in culture fluorescence accompanying ethanol exhaustion is clear.

In all applications of direct optical measurements in cell cultures, a number of potential problems arise which can interfere with interpretation of the measurements. For example, the optical surfaces in contact with the process fluid may become fouled with cells or medium components. Gas bubbles and particulates in the multiphase reaction fluid may interrupt or interfere with the desired measurement and, in the case of fluorescence, certain medium components or products may fluoresce at the same wavelengths useful for monitoring intracellular state, complicating interpretation of the measurements. However, in view of the importance of determining the biomass concentration and cellular metabolic state for monitoring, control, and optimization of the process, these and other optical methods can be expected to enjoy expanding applications in the future.

The following section is devoted to some of the intermittent, off-line analyses of cell and medium properties which are useful in bioprocess technologies. We should recognize that the dividing line between "continuous, on-line" monitoring and "intermittent, off-line" measurements is somewhat diffuse. If the time between successive off-line analyses is less than the characteristic time scale for changes in the measured quantity, then the intermittent, somewhat delayed

measurement is practically as useful as a continuous, real-time measurement. Since the time scales for changes in enzyme, cell culture, and reaction fluid properties may range from minutes to hours to days, certain off-line assays provide important information for process monitoring and control.

10.3 OFF-LINE ANALYTICAL METHODS

In this section we consider some of the measurement principles and methods applied to determine the properties of process fluids, biocatalysts, and biosorbants. Possible methods span the entire spectrum of analytical chemistry, spectroscopy, and biochemistry, making anything approaching a complete presentation impossible in this context. Here, we emphasize certain new methods relating to cell property measurements which have potential for process monitoring and control applications and also provide an overview of other types of commonly applied analyses.

10.3.1 Measurements of Medium Properties

After withdrawing a sample from a bioreactor or separation unit, a solid-liquid separation is accomplished by centrifugation or filtration in order to remove cells and any other particulate matter from the fluid phase sample. The analyses conducted subsequently, of course, depend upon the particular application; analytical methods which perform satisfactorily for defined medium may not be accurate or appropriate for analyses in undefined medium which may contain interfering components.

The desired measurements in a bioreactor are the concentrations of substrates and components influencing rates, and the concentrations of reaction products and inhibitors. For fermentation, analyses of the carbon and nitrogen sources are often desirable. Also, it may be necessary or useful to determine the levels of certain ions such as magnesium or phosphorus in the medium. Products of cellular processes vary over a broad range of chemical complexity and properties, from small organic compounds such as ethanol to more complex structures such as penicillin to biological macromolecules such as enzymes and other proteins. Accordingly, the spectrum of appropriate methods for product assay is extremely broad.

Liquid-phase quantitative analysis is based usually upon light refraction (measured with a refractive index detector), absorption of light at a particular wavelength (measured with spectrophotometer) or fluorescence due to excitation at one wavelength and subsequent emission at a longer wavelength (measured with a spectrofluorometer). Sugars, for example, do not absorb light strongly and do not fluoresce but do alter solution refractive index. On the other hand, protein and nucleic acids lend themselves to spectrophotometric and spectrofluorometric detection. Fluorescence measurements are usually more sensitive and allow measurement of lower concentrations. However, spectrofluorometers are more expensive than spectrophotometers, making spectrophotometric measurements very

popular. In order to avoid interference from other compounds in solution, separation or concentration of the component to be analyzed from other solutes is often necessary. Sometimes this can be accomplished by chemical treatment to decompose or to precipitate the desired interfering compounds. For example, RNA is extracted from cell lysates with $HClO_4$ (perchloric acid) at 37°C and analyzed by the orcinol method for ribose. Interfering sugars are removed during the extraction.

Finer-scale separation among related compounds by chromatographic methods is also commonly applied in medium chemical analysis. The basic principle of chromatography, which is discussed in greater detail in Sec. 11.4 below, is selective retention or retardation of certain compounds by an immobile phase in a column due to preferential attraction of these components for the immobile phase relative to other solutes. For example, in analyzing mixtures of sugars such as maltose and glucose, the different affinities for these two sugars for the primary amino groups on the surface of the support material in a commercially prepared carbohydrate column is used to separate the sugars in an HPLC (high performance liquid chromatography) apparatus. The different sugars emerge from the column at different times, and they may be then detected and quantified separately using a refractive index detector. Many other separations based on HPLC methods are useful in medium analyses. Also, separations accomplished under atmospheric pressure using ion exchange chromatography or size partitioning chromatography are useful in resolving mixtures of related components before their individual quantification.

As noted previously in Chap. 4, a useful strategy for chemical analysis is selective conversion of the component of interest to a readily measurable product. This is the basis for one of the standard laboratory methods for glucose assay, in which the enzymes glucose oxidase and peroxidase selectively convert glucose to a colored compound which can be assayed spectrophotometrically.

$$\text{Glucose} + 2H_2O \xrightarrow[\text{oxidase}]{\text{glucose}} \text{gluconic acid} + 2H_2O_2$$

$$H_2O_2 + o\text{-dianisidine (colorless)} \xrightarrow{\text{peroxidase}} \text{oxidized } o\text{-dianisidine (brown)}$$

Ion-specific electrodes have become important tools in assaying certain biologically important ions. Cellular nitrogen content can be determined with an ammonia electrode either directly (NH_3, or after chemical modification by pH adjustment, NH_4^+), reduction (NO_2^-, NO_3^{2-}), digestion (amino acids), or enzymatic modification (urea). Ion specific electrodes are also available for analysis of many other ions which influence biochemical structure and function including potassium, sodium, and calcium.

Occasionally, as an alternative to determining the concentration of a particular compound in solution, the measurement determines the compound's biological activity. Assay of penicillin in fermentation broths by this method has been a standard procedure in the pharmaceutical industry. Here, the size of a zone of dead bacteria around a porous disc soaked with the solution to be assayed

provides an indication of penicillin activity in the solution. It is very common to analyze enzyme content by measuring the activity of that enzyme. This functional assay is accomplished by exposing the sample solution to a standard enzyme substrate under standard conditions, then measuring the rate of substrate disappearance, or product appearance, often spectrophotometrically or by fluorescence.

An alternative set of analytical procedures is based upon volatilization of the components of interest and their measurement in the gas phase. This can be done for glucose, for example, by forming its TMS (trimethysilyl) derivative which can be vaporized in the injection chamber of a gas chromatograph and the product detected by a flame ionization detector. Determination of the contents of relatively volatile components such as ethanol, acetone, and butanol in fermentation fluids by this method is quite straightforward.

Analytical laboratories which support pilot- and production-scale fermentation facilities often contain one or more automated wet chemical analyzers. These automatically partition, dilute, and process a sample to carry out several chemical analyses. The response time of such instrumentation is 10 to 30 minutes, sufficient in many cases to be useful for monitoring of bioreactions in progress.

10.3.2 Analysis of Cell Population Composition

Analytical methods for cell populations can be categorized in much the same way as were mathematical models for cell population kinetics in Chap. 7 (recall Fig. 7.2). Most classical measurements of biochemistry provide population-averaged and thus unsegregated data on the cell population. Measurements of this type can be extended to a very large number of cellular constituents, even to the level of particular proteins, RNA molecules, and DNA molecules and sequences. If the experimental measurement is made on a single-cell basis, or can be used to infer single-cell information, so that the distribution of single-cell properties is obtained, the data may be said to be segregated.

We shall first discuss measurements of nonsegregated type. The most coarse of such measurements, after determination of total cell mass or number density, is analysis of the elemental composition of the cells including carbon, hydrogen, and nitrogen. Automatic analyzers such as the apparatus for determination of total nitrogen have been developed for bulk sample assays of this type. Specific ions are also known to play an important role in biological processes, and it is common to see total levels of iron, magnesium, phosphorus, and calcium reported as well. Determination of total protein, total RNA content, total DNA content, and other average macromolecular content of the cells can be accomplished by well-established methods. Shown, for example, in Fig. 10.9 are some of the procedures useful in analysis of the macromolecular composition of yeast. It is beyond the scope of this text to describe each of the individual analytical methods for each class of macromolecules in detail; they can be found in Refs. 14 and 15 as well as in many research publications dealing with composition measurements during cellular growth. Measurements of this type provide the

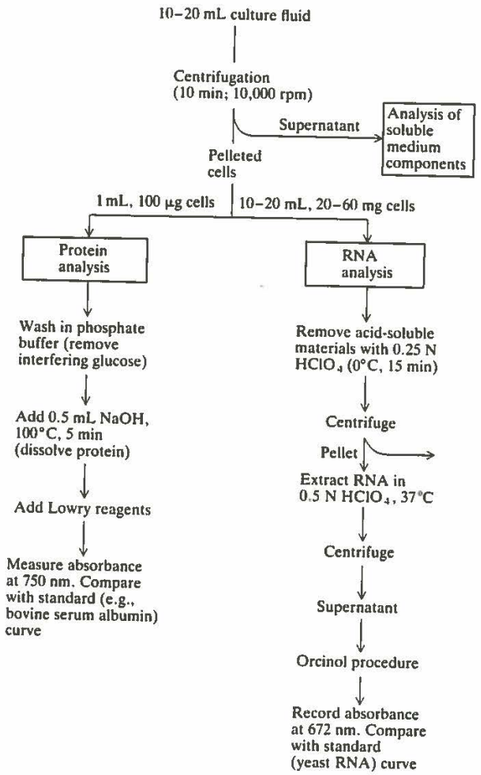

Figure 10.9 Flowchart of analysis procedures for determination of the (population-average) protein and RNA content of yeast cells. Details on the Lowry and orcinol procedures may be found in Refs. 14, 15.

experimental basis for results such as those shown in Fig. 7.17. Although a given method of protein or DNA analysis, for example, will often apply for many different types of organisms, it may be necessary to adapt analytical methods to the particular species under investigation.

Population-average cell content of particular proteins can be determined in several different ways. First, for enzymes, activity assays are used to monitor the

Table 10.3 Characteristic substrates and measurement principles for assay of activity of key enzymes in the catabolic pathways of *Clostridium acetobutylicum*

Enzyme	Substrate	Reaction	Measurement
Acetoacetate decarboxylase	Acetoacetate	Acetoacetate $\longrightarrow$ acetone + CO_2	Consumption of acetoacetate by spectrophotometry ($\Delta OD_{270\,nm}$)
Phosphofructokinase	Fructose-6-phosphate	Reaction coupled with aldolase, triosephosphate isomerase, and α-glycerophosphate	Consumption of NADH by fluorometry (excitation 340 nm, emission 460 nm)
Hydrogenase	Methylviologen	Dehydrogenase methylviologen + $2H^+ \longrightarrow$ methylviologen (ox) + H_2	Oxidation of methylviologen which produces H_2 gas is followed manometrically
Pyruvate-ferredoxin oxidoreductase	Acetyl-CoA	$^{14}CO_2$ + acetyl-CoA + ferredoxin(red) $\longleftrightarrow$ ^{14}pyruvate + CoA + ferredoxin(ox)	Production of pyruvate by scintillation counter (radioactivity of ^{14}C)
Butyrokinase	Butyrophosphate	Butyrophosphate + hydroxylamine $\longrightarrow$ butyrohydroxamic acid + $HOPO_3^-$	Production of butyrohydroxamic acid by spectrophotometry (ΔOD at 540 nm)

changes in enzyme levels during process operation. Table 10.3 lists the characteristic reactions and measurement principles involved in assaying levels of key enzymes in the metabolic pathways from glucose to organic solvents in the bacterium *Clostridium acetobutylicum* used for fermentative production of acetone and butanol. Shown in Fig. 10.10 are the time courses of activity of several key enzymes in this organism during batch cultivation. Here it is seen that the activity levels of the enzymes associated with acids production decline late in the fermentation, while there is an increase in enzyme activity associated with solvents production late in the batch. Based upon information of this type, alterations in metabolism may be more directly correlated with strain and bioreactor operating parameters in order to optimize the organism and the process conditions.

Individual proteins can sometimes be analyzed by protein chromatography (see Sec. 11.4) or by examining the relative intensities of bands obtained by electrophoresis of a protein mixture. Increased resolution and extreme sensitivity to many different protein levels can be achieved by two-dimensional gel electrophoresis in which proteins are separated in one direction on the basis of their size

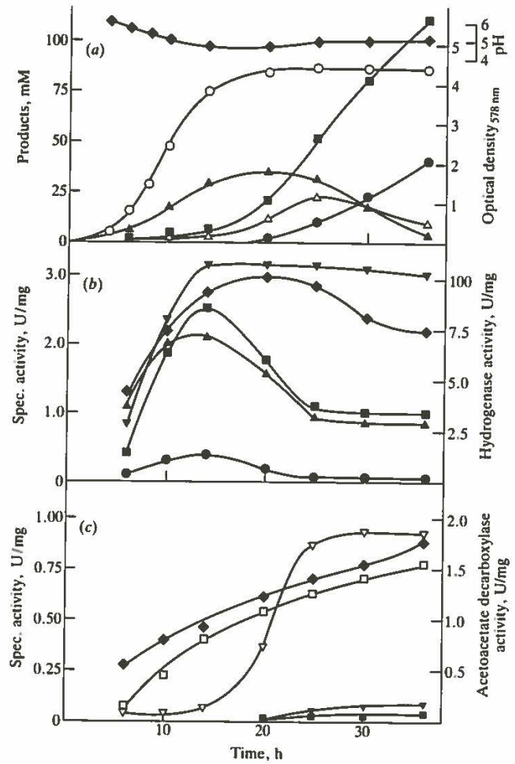

Figure 10.10 Growth of *Clostridium acetobutylicum* in batch culture and level of enzymes involved in product formation. (a) Growth parameter and products formed. Optical density, ○; pH-value, ◆; acetate, △; butyrate, ▲; acetone, ●; and butanol, ■. (b) Level of enzymes involved in the formation of acetate and butyrate and of hydrogenase. Phosphotransacetylase, ●; acetate kinase, ■, phosphotransbutyrylase, ◆; butyrate kinase, ▲; and hydrogenase ▼. (c) Level of enzymes involved in the formation of acetone and butanol. Acetoacetyl-CoA: CoA-transferase with acetate, (◆) or butyrate (□) as acceptor; acetoacetate decarboxylase, ▽; butyraldehyde dehydrogenase, ■; and butanol dehydrogenase, ▼. *(Reprinted by permission from W. Andersch, H. Bahl, and G. Gottschalk, "Level of Enzymes Involved in Acetate, Butyrate, Acetone and Butanol Formation by* Clostridium acetobutylicum," *Eur. J. Appl. Microbiol. Biotechnol., vol. 18, p. 327, 1983.)*

and in a second direction on the basis of their charge. When separated in this way, different proteins tend to move to different spots in the two-dimensional plane, making it possible to identify and quantify a large number of proteins simultaneously. For example, this method was used to study the rates of synthesis of 140 different proteins during growth of the bacterium *E. coli* [17].

Another basis for analysis of individual proteins is binding of antibodies to a particular region on an individual protein molecule [18]. If antibody is available for the protein of interest, analyses based upon precipitation, detection of radioactively labeled antibodies, or the amount of enzyme activity which can be linked to a particular protein by an antibody [the enzyme-linked-immunosorbent assay (ELISA) method] may be used to quantify the amount of the individual protein present. Such methods may also be applied to analyze cellular content of other components or of macromolecular structures against which specific antibodies can be made. Antibody labels are used frequently to determine the existence on a cell surface of particular types of molecules or structures and to quantify in some cases the amount of these components on the cell exterior. Labeling of cell surface compounds and subsequent measurement may be conducted without killing the organisms, a feature which may be useful in screening or selection during strain improvement by mutation. Such methods are also convenient for distinguishing between species in a mixed culture, since organisms usually carry specific surface markers which can be identified separately and quantified with specific antibodies.

The importance of plasmids as the carriers of the genetic instructions for product synthesis in recombinant organisms makes assay of cellular plasmid content a potentially important measurement. The most rigorous method of plasmid quantification in bacteria is done by isolating all DNA from the organism, then separating plasmid DNA from chromosomal DNA in a cesium chloride gradient using an ultracentrifuge. The relative quantities of chromosomal and plasmid DNA can be examined in several ways. For example, if a radioactive preparation of DNA was used, fractions can be collected from the bottom of the tube and analyzed for radioactivity using a scintillation counter. Determination of plasmid DNA content in yeast or animal cells may be accomplished by a hybridization assay using a labeled probe complementary to a nucleotide sequence unique to the plasmid. Alternatively, the gene for a particular enzyme, the activity of which is easy to assay, may be included on the plasmid as a marker, and the activity of this enzyme used to estimate the plasmid content of the organisms. This latter method has been implemented in bacteria, yeast, and animal cell recombinant strains.

All of the measurements discussed above provide information on cellular composition and to some degree on tendencies in metabolism, but they do not directly indicate the current metabolic state or energetic state of the organism. Measurement of cellular ATP content can be carried out with a Biometer that measures luminescence produced by a reaction requiring ATP and catalyzed by the enzyme luciferase. Since ATP levels change rapidly as a function of cellular environment and metabolic activity, it is necessary that samples of the cell population be quenched rapidly in phosphoric acid in order to preserve their ATP content before this or alternative ATP analyses. Since ATP is absent from nonviable cells, measurements of the ATP level can also be interpreted usefully in some cases as a measure of the metabolically active biomass in the population.

High resolution nuclear magnetic resonance (NMR) measurements of ^{31}P have been successfully applied to determine intracellular ATP, ADP, sugar phosphate, polyphosphate, and pH values. Several different microorganisms have been studied in this fashion including the bacteria *E. coli* [19] and *Clostridium thermocellum* [20] and the yeasts *Saccharomyces cerevisiae*, *Candida utilis*, and *Zygosaccharomyces bailii* [21]. In addition, tracer isotopes such as ^{13}C and ^{15}N may be used to observe functioning of intracellular pathways of carbon and nitrogen metabolism via NMR [23, 24].

It has been observed in several fermentations with mycelial microorganisms that the process productivity and kinetics are correlated with the morphological state of the mold or actinomycete. Example 7.2 presented some data and discussion of the connection between morphology and product formation for cephalosporin production using the mold *Cephalosporium acremonium.* Direct observation and quantitative monitoring of mycelial morphology is quite difficult and time-consuming since repeated microscopic observations and human or computerized image analysis are involved. It has been observed that the filtration properties of a suspension of mycelia are influenced by the mycelial morphology, and this principle has been used by Wang and collaborators [25, 26] and refined by Lim and colleagues [27] to formulate an ingenious mycelium morphology and biomass probe based upon a batch filtration measurement. A small sample of culture suspension is filtered, and the filtrate volume and cake thickness are monitored continuously. Based upon previously established correlations between filtration characteristics and the morphological properties and density of the particular mold considered, these data provide a basis for intermittent on-line monitoring of the progress of the fermentation. (see Prob. 11.6)

There are several different methods available for measuring and characterizing the distribution of single-cell characteristics in a population of single-celled organisms. Microscopic observation can give some approximate indications and, coupled with image analysis methods, quantitative information can be obtained, although gathering data on a sufficiently large number of cells to have a good statistical sample is rather difficult. More suitable for rapid measurements of properties of large numbers of individual cells are flow measurement methods of which there are two general types. In instruments utilizing the Coulter principle, the volume of individual cells is detected as cells suspended in a sample stream of an electrolyte solution flowing through a small orifice across which resistivity is measured. For spherical particles, the alteration in resistivity across the orifice may be correlated directly with the volume of the spherical particle, allowing many particles to be sized as they flow rapidly through the orifice. Alterations in particle morphology can cause some difficulties in interpretation of the measurements, but still this is a useful approach for obtaining the size distribution in a cell population.

A richer class of measurements is possible using a flow cytometer. In this instrument, a dilute cell suspension again flows through a measuring section and, in this case, optical measurements are conducted. As indicated in the schematic diagram of Fig. 10.11, the cell sample stream is irradiated by a laser or other light source and the light absorption, scatter and or fluorescence is measured on a single-cell basis. Light-scattering measurements may be used to obtain information on the cell size distribution. Since right-angle light-scattering intensity is sensitive to intracellular morphology, this measurement has been applied to monitor the accumulation in individual bacterial cells of refractile particles consisting of the storage carbohydrate polyhydroxybutyrate [28]. Individual cell macromolecular composition has been measured for microorganisms and animal cells by applying specific fluorescent dyes which label the macromolecular pool of interest including total cellular protein, double-stranded RNA and cellular DNA [29, 30]. Accumulation of an intracellular fluorescent product produced under the action of a single enzyme can be monitored on the single-cell level in such an instrument, allowing assay of individual enzyme activity in individual cells, study of *in vitro* enzyme kinetics and, by cloning the gene for this enzyme on a plasmid, characterization of single-cell plasmid content [31]. Flow cytometry measurements may also be used to differentiate and quantify multiple species in a mixed culture and to detect the presence of contaminant in a fermentation inoculum [32, 33]. Since flow cytometry provides not only average information but gives a distribution of single-cell characteristics in the population, the data is rich and provides detailed insight into the state of the microbial population.

Although most applications of flow cytometry to study fermentation processes have involved single-parameter measurements, it is possible to effect simultaneous multiple measurements on individual cells, gaining even further detailed information on the cell population. Data of this type, considering two-parameter measurements as an example, take the form of a surface indicating frequency or relative number of cells as a function of the coordinates in an underlying plane representing the measured quantities. Figure 10.12 shows example data of this type in which the measured single-cell properties are light-scattering intensity related to cell size and intracellular fluorescence produced by the action of an enzyme encoded on a plasmid gene. Thus, the amount of fluorescent product accumulated may be correlated with the existence and even with the number of plasmids in the yeast cell. The data in Fig. 10.12 show two clear peaks which represent different types of cells in this culture. Those with relatively small fluorescence intensity are cells without plasmids which exhibit a low degree of natural fluorescence under the measurement conditions applied. The population with larger fluorescence, evident as a distinct mode or mountain in this sort of measurement, represents cells containing plasmid and therefore the enzyme required to generate an additional fluorescence response. Based upon this type of measurement, the proportion of cells with and without plasmid in the culture can be very rapidly assayed and further information can be extracted on plasmid replication and segregation in the recombinant strain.

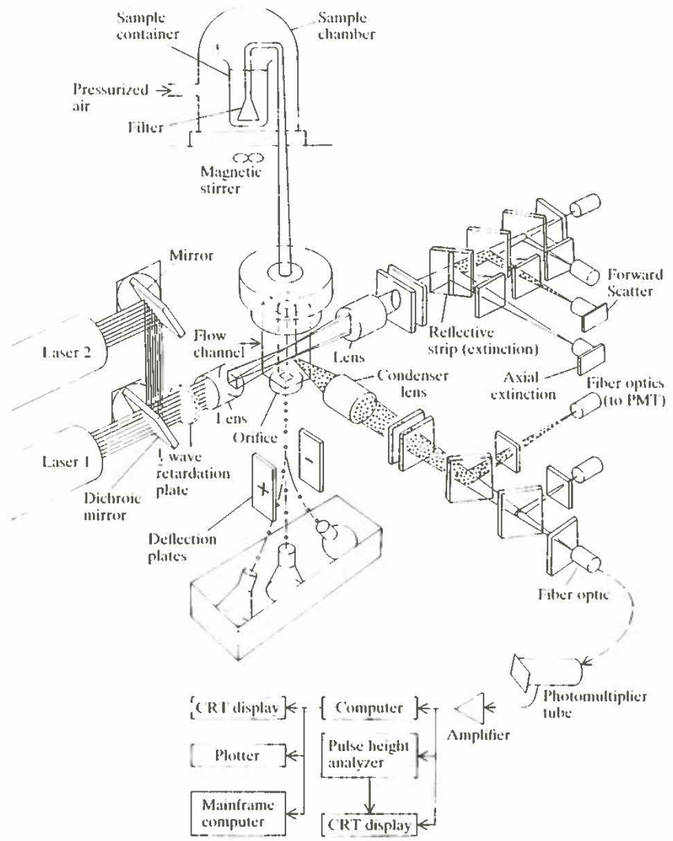

Figure 10.11 Schematic diagram of a flow cytometer showing the paths of the flowing suspended cell sample, the laser beams, optical filters and detectors for measuring single-cell properties, and the electronic signals generated, analyzed, and stored. Diagnonally placed square elements are dichroic filters (reflect certain wavelengths and pass others), and orthogonally placed elements are barrier filters based on wavelength, polarization, etc. Many instruments contain multiple photomultiplier tube detectors and associated electronics, permitting multiple simultaneous measurements on each cell. *Based on Ortho Instruments Cytofluorograph System 50H.*

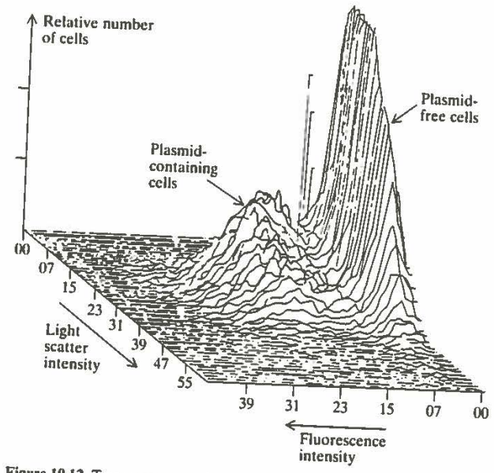

Figure 10.12 Two-parameter characterization of a population of recombinant *Saccharomyces cerevisiae* by flow cytometry. Axes of the basal plane denote single-cell light-scattering intensity (correlated with cell size) and single-cell fluorescence intensity which here is correlated with cellular plasmid content. Here, plasmid-free and plasmid-containing cells are readily distinguished based on fluorescence.

10.4 COMPUTERS AND INTERFACES

There are a number of advantages to be gained by coupling process instruments to digital computers. First, the computer can enhance *data acquisition* functions in several respects. Improved reliability and accuracy can be obtained by using statistical methods and digital filtering. Readings from several parallel sensors can be compared and analyzed to provide on-line recalibration and to identify sensor failure. With a computer, the number and sophistication of analysis systems can be increased. For example, a computer-controlled system may take samples automatically, conduct a chromatographic analysis, and interpret the results, using internally stored calibrations or algorithms to give output directly in convenient units. Although simple signal conditioning and correcting operations such as linearization can be done with particular electronic circuits, these functions are readily accomplished using a computer without the need for additional specific hardware. Another advantage of computers with respect to data acquisition is the ability to store large quantities of measured results in digital form which may be accessed conveniently, analyzed, and displayed later.

Using computers, *data analysis and interpretation* can be enhanced greatly. Results of several measurements may be combined to calculate instantaneously quantities such as oxygen utilization rate and respiratory quotient. Advanced state and parameter estimation methods may also be applied on-line to provide additional useful information on process status from the limited measurements available. More specifics and some examples of computer applications for data analysis are presented in the next section.

Computers expand opportunities tremendously for *improved process control and optimization.* One computer can replace many conventional analog controllers and control many individual valuables such as pH and temperature using standard feedback algorithms. Furthermore, more sophisticated multivariable control methods may be implemented easily with a computer. Controlled variables may include derived quantities such as RQ when a computer is applied. Computer methods may be used to evaluate and improve process mathematical models which may then be employed for determining optimum operating conditions and strategies. Then, the computer provides the memory and computation capability to implement the optimization method, such as variation of nutrient feeding rate or pH during a batch fermentation.

Operation of a batch process requires a carefully controlled and coordinated sequence of valve openings and closings and pump starts and stops. While all of these functions have been done by various timers and relays in earlier technology, they may now be managed efficiently by computer. Use of a computer to manage such switching operations during batch process operation becomes essential if we wish to optimize the scheduling of a number of parallel batch processes (e.g., fermentors) which feed sequentially to downstrean batch processes (e.g., precipitation, chromatography, and so forth). We shall examine elementary process regulation and more ambitious control objectives and strategies in Sec. 10.6 and 10.7, respectively.

Before turning to these interesting domains of computer application, we shall examine briefly some of the principles of digital computers and computer interfaces. Our objective here is to introduce some generic concepts and, by example, to illustrate specific realizations of different types of computer-process configurations. Because improvements and cost reductions in computer hardware and software are proceeding presently at a rapid rate, any specific computer system is probably outdated by the time its description has been published—certainly in book form. Thus, we should view the examples here and in the remainder of the chapter as the kinds of things which can be done, recognizing that, as of this reading, there are probably cheaper, more efficient ways of doing the same thing or something even more effective.

10.4.1 Elements of Digital Computers

The basic components of a digital computer are shown in the block diagram in Fig. 10.13. The central processing unit (CPU) accepts instructions from a stored program through its control unit and performs the indicated arithmetic and

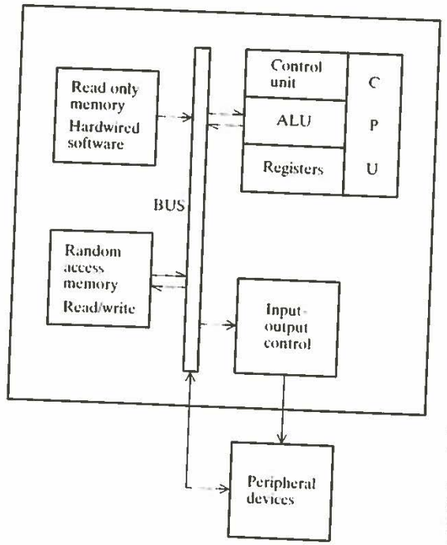

Figure 10.13 Basic functional elements in a computer. CPU denotes central processing unit and ALU stands for arithmetic and logic unit. The different elements communicate through a bus system. (*Adapted from H. A. Hampel, "Application of Microcomputers in the Study of Microbial Processes," p. 1, in "Advances in Biochemical Engineering, Vol. 13," [T. K. Ghose, A. Fiechter, and N. Blakebrough (eds.)], Springer-Verlag, Berlin, 1979.*)

logical operations in the arithmetic and logic unit (ALU), using internal registers for short-term storage. Operations in the CPU are controlled and synchronized by an internal quartz oscillator clock. The cycle time of the CPU, which may range from less than 10 to 10^4 ns (10^{-9} s), combined with a number of bytes (each byte contains eight bits, a binary number with value 0 or 1) processed per cycle (the word size), determines the speed of computation in the CPU. For example, microcomputers available in 1980 employed 8-bit words. By 1982 16-bit microcomputers were available, and 32-bit machines were manufactured by several companies in 1984.

Memory for storage of program instructions and data is provided in several different forms. Read-only memory (ROM) contains fixed instruction sets such as compilers and interpreter programs, while random access memory (RAM) is used for short-term storage of programs, input data, and computational results. The CPU reads frequently from and writes on the RAM during computer operation. As of the mid-1980s, popular microcomputers had RAM capacities from 64,000 bytes (64 kilobytes or just 64 K) to 512 K and beyond. Additional memory is usually available also in one or more peripheral devices. Common external mass memory devices for use with microcomputers are magnetic tape cassettes (storage 80–300 K, access time ~10 s), floppy disks (100–600 K, access time ~0.5 s) and hard disks (500–20,000 K, access time ~3 s; all numbers as of 1984).

The input-control allows the computer to communicate with external peripheral devices. Within the computer, a bus system interconnects the CPU, memory, and input-output control segments.

The functional elements described above are common to all computers, but the speed and memory capacity are determined by the particular hardware configuration. Based on these parameters, computers are often classified into supercomputers, mainframe computers, minicomputers, and microcomputers, with this list ordered from largest, fastest, and most costly to smallest, slowest, and least expensive. Definition of the boundaries between these different classes of computers is constantly shifting; today's microcomputers have the power of mainframe computers of the 1970s. The availability of tremendous computing capacity at low cost is driving a revolution of new computer uses in consumer products, communications, information processing, scientific instrumentation, and in biotechnology. While computer-coupled fermentors were a novelty in the 1960s, we can expect in the not too distant future that almost every bioreactor, analytical instrument, and other bioprocess unit will be monitored and controlled by digital computers.

10.4.2 Computer Interfaces and Peripheral Devices

The storage and arithmetic and logic capabilities of a computer are worthless unless the computer is connected to or interfaced with something else. We can classify computer interfaces as follows according to the object connected with the computer:

1. Computer → computer: communication
2. Operator → computer: instruction
3. Computer → operator: information
4. Sensor → computer: input
5. Computer → actuator: manipulation

We shall next consider each of these types of interface and their significance in computer applications in biochemical engineering. Before turning to particular interface classes, however, we should make the general comment that communications hardware and software controls applied to a particular type of interface are not standardized, so that different computers, peripheral devices, and sensors cannot be "plugged-in" to each other arbitrarily. Care must be exercised to ensure that units to be interconnected have compatible input-output functions.

Computer-computer connections are important because different types of computers and digital devices have different costs and capabilities. Overall efficiency is maximized and cost minimized by using the minimum computing power for the major task at hand, communicating with a higher-level computer when more rapid or complex computational operations or larger storage are needed. A proposed hierarchy of computing levels applicable to fermentation research and development is shown in Fig. 10.14. Here, at the first and lowest level, which

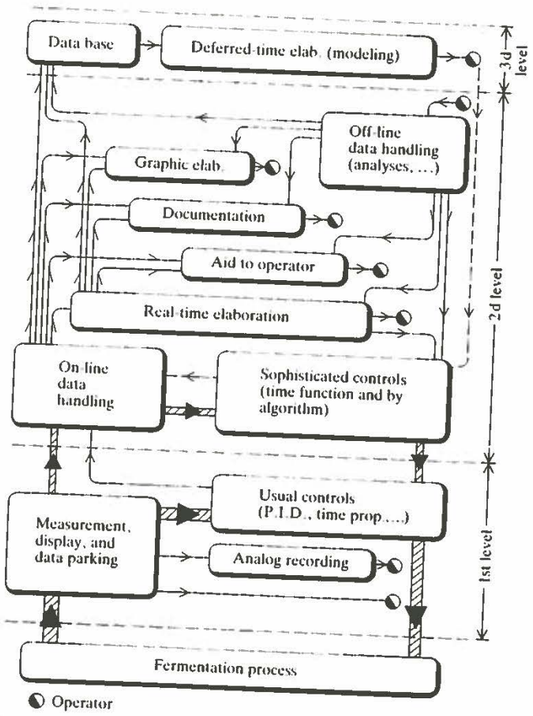

Figure 10.14 A useful breakdown of functions in a hierarchical computer system. Level 1, 2, and 3 functions correspond approximately to microcomputer, minicomputer, and mainframe computer hardware, respectively. (*Reprinted by permission from L. Valentini, E. Andreoni, M. Buvoli, and P. Pennella, "A Computer System for Fermentation Research: Objectives, Fulfillment Criteria and Use," p. 175 in "Computer Applications in Fermentation Technology," Society of Chemical Industry, London, 1982.*)

could be handled by a microcomputer, measurements from the fermentation process are displayed and stored and conventional single-loop regulatory control functions (see Sec. 10.6) are provided. A mini-computer might manage the second level where more detailed data analysis and further operator interaction occurs. Also, the calculations needed for more sophisticated control algorithms could be done at this level.

The first- and many of the second-level functions are conducted in *real time*, which means that there is negligible lag between input of information or instructions to the computer and output or control action based on that information. At the highest or third level, a mainframe computer may be used to solve complicated model equations, estimate model parameters from obtained data, and evaluate advanced control and optimization strategies. Here, information supplied from the first- and second-level computers would be used, but the results would not necessarily be obtained in real time. Instead, the goal may be improvement of process operation at some future time.

Communication at the "person-machine interface" between operator and computer takes several different forms. Computer-to-operator communication may occur using a digital meter, a CRT (cathode-ray tube display), printer, plotter or, recently, computer-generated speech. The form of output displayed on a CRT printer or plotter may be text or graphical format shown in single or several colors. Operator input to the computer is often using a keyboard, directly communicating with the computer in the case of a terminal or indirectly using a keypunch. Other modes for operator input include switches, touch-sensitive screens, and "mouse" devices which, when rolled on a hard surface, translate correspondingly a cursor on a CRT display. Also, speech recognition capabilities, the object of intensive contemporary R & D activities, already permit a small set of instructions to be entered verbally. Input of prerecorded programs or data is done through one of the mass memory peripherals mentioned earlier.

The convenience and flexibility of operator-computer interactions combined with the computer's ability to store and manipulate rapidly large quantities of data have several significant benefits in biotechnology as in other areas of process technology. Process and controller status can be communicated to the process operator in clear, efficient fashion, giving the operator the option of examining particular aspects of the process in greater detail on command. Alarms and cues generated by the computer can alert the operator to existing or emerging problems in the process. Also, the operator possesses great flexibility in altering controller settings or, if needed, even the controller algorithms and configuration.

In addition, safety and regulatory policies usually require gathering and maintaining detailed records on each batch of a drug. Such record-keeping and report generation is facilitated greatly if a computer is linked to the process. Reports on raw material and energy consumption, cycle times, yields and inventories important in R & D, in plant management, and in overall economic optimization for the company may be prepared more rapidly and with much less effort if the process units are computer-coupled. Benefits solely from improved record-keeping and report generation have provided the economic justification for installation of process computers in several pharmaceutical companies.

Sensor-to-computer interfaces depend on the measured quantity and the available forms of sensor output. Some important process operating variables have only "on" or "off" states such as running of a pump or compressor or an open or closed valve. These may be read by the computer directly in digital form. Another type of digital input is a pulse which is generated by a tachometer or

mass-flow meter for a given amount of shaft rotation or mass. Rates are then determined by counting pulses per unit time interval.

Many sensors monitor parameters which have a continuous range of values. Traditionally, in addition to an analog meter readout on the measuring unit, an analog current or voltage output is provided for input to a stripchart recorder or perhaps an electronic controller. These analog signals may be input to a computer through an *analog-to-digital* (A/D) *converter*. Usually, the analog signal provided by the analysis instrument must be conditioned suitably that is, translated, amplified, or attenuated and perhaps converted from current to voltage before it is input to the A/D converter which is designed to accept analog

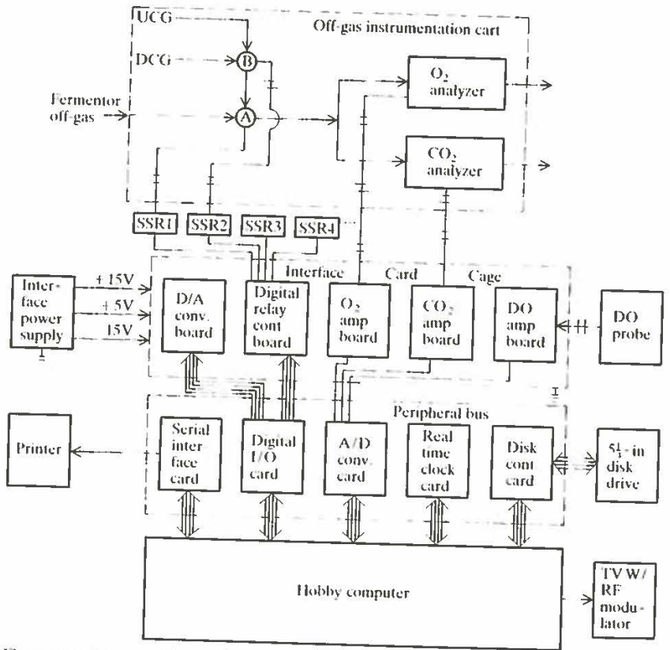

Figure 10.15 Schematic diagram of a gas analysis system based on a microcomputer. UCG and DCG denote upscale and downscale calibration gases which may be introduced intermittently into the gas analyzers in place of fermentor off-gas. This switching is controlled by the computer through solenoid switch relays (SSR1, etc.). *(Reprinted by permission from E. H. Forrest, N. B. Jansen, M. C. Flickinger, and G. T. Tsao, "A Simple Hobby Computer-Based Off-Gas Analysis System," Biotech. Bioeng., vol. 23, p. 455, 1981.)*

signals over a prespecified range. Resolution of the analog signal is directly related to the number of bits employed. If the digital output has n bits, the input analog range will be divided into 2^n discrete subintervals by the converter.

An example of a laboratory data acquisition system based on a microcomputer is illustrated schematically in Fig. 10.15. As mentioned, the O_2 and CO_2 analyzer analog outputs are adapted before going to the A/D converter. Interestingly, notice that an amplifier board has been used to input directly the voltage generated by the dissolved oxygen probe without the use of a separate instrument or readout unit for DO. By replacing some of the functions of analog instruments and controllers, a microcomputer system can provide all of the advantages discussed at lower cost than the traditional set of analog instrumentation.

Two different approaches have been used in connecting several analog inputs to a computer. The first uses a separate A/D converter for each input (there are usually several such converters on a single A/D board). Alternatively, a scanning unit containing several relays (relay multiplexer) may be used to switch among several analog inputs, feeding the selected input to a single A/D converter. The former approach permits more rapid sampling and reading of each analog input at the cost of a larger number of A/D converters. Increasingly, analysis instruments are being equipped with digital outputs, usually in the form of a *binary coded digital* (BCD) *signal*, which may be used for computer input.

Consideration of computer-to-actuator interfaces parallels that just described for physical device-to-computer communication. Digital outputs control electrical on/off switches (relays) and stepping motors. *Digital-to-analog* (D/A) *converters* send to actuators analog signals corresponding to digital value output of the computer. Figure 10.16 shows the interconnections and interfaces used in computer coupling to a pilot plant. While the complexity and scope of the two systems clearly differ, the major components, types of information, and signal flows are very similar to those in the laboratory gas analysis microcomputer module in Fig. 10.15.

10.4.3 Software Systems

The *software* the set of programmed instructions which govern the operation of the computer, its interfaces and its peripheral devices is a critical component of a computer-coupled fermentation process. The software dictates how the computer, and perhaps ultimately the process, will perform. Which data is displayed and stored in what format, what operator inputs or interventions are necessary or allowable, perhaps how the process is operated, are determined by the software. It has long been recognized by those with experience in the field of computer-coupled instruments and processes that good software is a central key to a successful system, and that software development can be the major task and a significant expense in installing a process computer. Recent downward trends in hardware prices certainly reinforce this theme. Consequently, it behooves the laboratory researcher and plant manager alike to examine carefully the

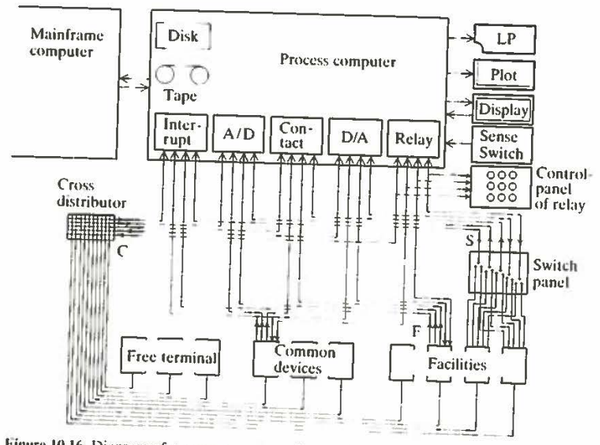

Figure 10.16 Diagram of a computer system for monitoring and control of a fermentation pilot plant. *Abbreviations:* LP, line printer; F, fixed wiring; S, switching system; C, cross distributor. Contact sensors receive information on measurement equipment and input from the fermentor operator. *(Reprinted by permission from M. Meiners and W. Rapmundt, "Some Practical Aspects of Computer Applications in a Fermentor Hall," Biotech. Bioeng., vol. 25, p. 809, 1983.)*

capabilities and flexibility of existing software for alternative hardware and to consider carefully the ultimate costs of not paying what may seem initially like a high price for system software.

The *operating system* of the computer controls program execution, file storage management, inventory and allocation of memory, and coordination of these functions. There are three different types of computer programs: *utility programs* which start up the system and create files, *language programs* to permit use of high level languages (BASIC, FORTRAN, APL and others), and *applications programs* for accomplishing particular computations and other tasks. Some objectives of applications programs such as data acquisition, operator information, and report generation have already been discussed, and others including data handling and process control are considered in the remainder of this chapter. Examples of particular algorithms and application program functions abound in the chapter references.

An important consideration in selecting a hardware-software system is its ability to do time-sharing or multitask operations. With this capability, the computer system essentially can run several programs simultaneously, thereby observing, analyzing, and controlling several different process units at the same time. Also, new programs can be written and old ones debugged or modified while the computer continues to interact with one or more processes. Multitask

capability is essential at the second and third levels displayed in Fig. 10.14, but the dedicated microcomputer data acquisition and analysis system in Fig. 10.15 does not require time-sharing features.

Having surveyed the basic concepts important in process computer systems, we now examine some uses of computers and of data which they have acquired to define the operating state of the process and to provide the desired environmental conditions for maximizing bioprocess productivity.

10.5 DATA ANALYSIS

Although the available measurements for a bioreactor are limited, these can be used in concert with defining equations, overall mass and energy balances, and process mathematical models to deduce the values of process variables and parameters. As A. E. Humphrey suggested in 1971, the available measurements taken together provide a "gateway" into other aspects of the process which are not directly observed. We have already discussed how measurements of inlet and exit gas flow rates and composition may be used to calculate the average volumetric mass-transfer coefficient, $k_l a$, for a bioreactor (see Sec. 8.2.1). Also, in Sec. 5.10 we saw how macroscopic balances on cellular growth could be used systematically to calculate macroscopic flows based upon known overall cellular reaction stoichiometry. Figure 10.17 shows in flowchart form how measured quantities may be used to calculate related process properties associated with mixing and aeration and with cell growth and metabolism.

On-line estimation of biomass concentration and of specific growth rates during fermentation has been a central objective for data analysis methodologies. In this section, we will use this biomass estimation problem as a prototype for illustration of several different approaches to bioreactor data analysis.

10.5.1 Data Smoothing and Interpolation

Often, measurements obtained from process instruments are noisy. Significant measurement fluctuations make the instrument outputs unsuitable for use as an accurate, instantaneous value. Some sort of signal conditioning or smoothing is required in such cases.

The simplest way to smooth data is to apply a first-order (analogous to an RC) filter which attenuates input fluctuations smoothly and increasingly as the fluctuation frequency increases. Such a filter is characterized by a time constant t_f: inputs with frequencies much smaller than t_f^{-1} are attenuated to negligible magnitude in the output, and fluctuations with frequencies much greater than t_f^{-1} pass essentially unaffected. In discrete-time form, convenient for digital computer implementation, such a filter is given by [2]

$$w_k = a_0 u_k + a_1 u_{k-1} - b_1 w_{k-1} \tag{10.4}$$

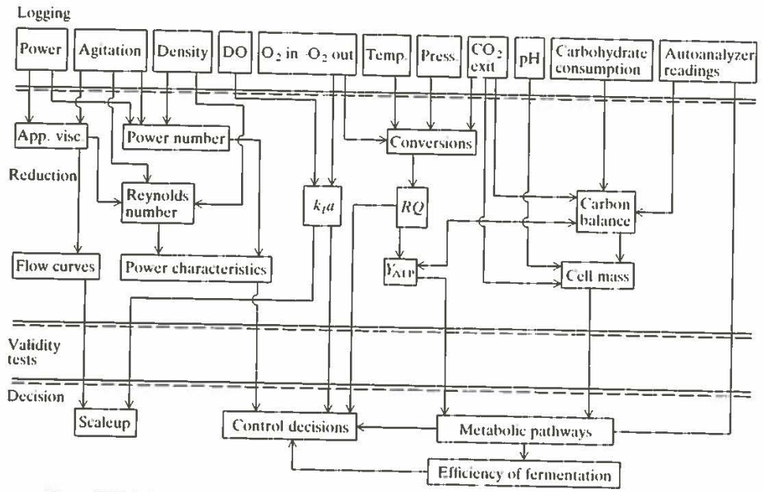

Figure 10.17 Primary measurements, shown on top, may be used to calculate many related process properties and parameters. *(Reprinted by permission from L. P. Tannen and L. K. Nyiri, "Instrumentation of Fermentation Systems," p. 331 in "Microbial Technology," 2d ed., Vol. II [H. J. Peppler and D. Perlman (eds.)], Academic Press, New York, 1979.)*

where u is the filter input (noisy signal), w is the filtered output, and subscripts k and $k-1$ denote the current and the previous discrete sampling times. a_0, a_1, and b_1 are parameters which determine the filter characteristics. In particular, t_f is related to the *sampling time* T_s (the time interval between samples) and the parameter b_1 by the equation

$$t_f = \frac{T_s(1 - b_1)}{2(1 + b_1)} \tag{10.5}$$

Somewhat simpler to implement, yet giving similar filtering characteristics, is a *moving average* calculation. Here, we take measurements more frequently, and average some set, say 10, of the sequence of measurements to obtain a representative value over the time for all of those measurements. This is quite feasible for bioreactions where significant changes in measured quantities often occur over a much longer time scale than the time required for the measurement.

A more sophisticated filter which also provides an interpolation polynomial has been proposed for fermentation applications by Jefferis and coworkers [35]. We shall consider estimation of biomass density and growth rate from noisy, intermittent turbidity measurements as an example. The cell mass density within

some time interval τ backward from the current sampling time is represented as a second-order polynomial in time:

$$x(t) = \alpha_1 + \alpha_1 t + \alpha_3 t^2 \tag{10.6}$$

The objective is to estimate values of the coefficients α_1, α_2, and α_3 using the present measured value of x, $x(t_k)$, previously measured values, and possibly information on measurement noise as well. One approach is to effect a least-squares deviations fit of Eq. (10.6) to the data over the time interval $t_k - \tau$ to t_k. A recursive least-squares filtering method for this purpose is described in Ref. 35. Other techniques for filtering noisy data are discussed in the general references at chapter's end.

10.5.2 State and Parameter Estimation

Neglecting accumulation of oxygen in the reactor, the oxygen material balance on a batch reactor becomes

$$\frac{F_f c_{O_2,f}}{V} - F_e c_{O_2,e} = Q_{O_2} = M_{O_2/X} x + \frac{1}{Y_{X/O_2}} \frac{dx}{dt} \tag{10.7}$$

where f and e denote feed and exit values, respectively. In this equation, oxygen use for both growth and maintenance metabolism is considered. By measurements on the feed and exit gas streams, the *oxygen utilization rate* Q_{O_2} (this quantity is often also indicated as OUR in the literature) may be determined experimentally. With $Q_{O_2}(t)$, a known function of time and assuming the coefficients $M_{O_2/X}$ and Y_{X/O_2} to be constants, Eq. (10.7) may be integrated to obtain

$$x(t) = \exp(-M_{O_2/X} Y_{X/O_2} t) \times \left[x(0) + \int_0^t Y_{X/O_2} \exp(M_{O_2/X} Y_{X/O_2} \tau) Q_{O_2}(\tau)\, d\tau \right] \tag{10.8}$$

This equation may now be used to estimate values of $x(t)$ from $Q_{O_2}(t)$ measurements. Then, the growth rate dx/dt and specific growth rate follow directly from Eq. (10.7).

However, before applying this to a particular fermentation, the oxygen stoichiometric parameters must be determined. A convenient equation for this purpose may be obtained by integrating Eq. (10.7) with respect to time and rearranging to obtain [29]

$$\frac{\int_0^t Q_{O_2}(\tau)\, d\tau}{\int_0^t x(\tau)\, d\tau} = M_{O_2/X} + \frac{1}{Y_{X/O_2}} \left[\frac{x(t) - x(0)}{\int_0^t x(\tau)\, d\tau} \right] \tag{10.9}$$

Using measured values of $Q_{O_2}(t)$ and $x(t)$ from an off-line experiment, the parameters $M_{O_2/X}$ and Y_{X/O_2} may be determined from a linear plot of the left-hand side of Eq. (10.9) vs. the bracketed quantity on the right.

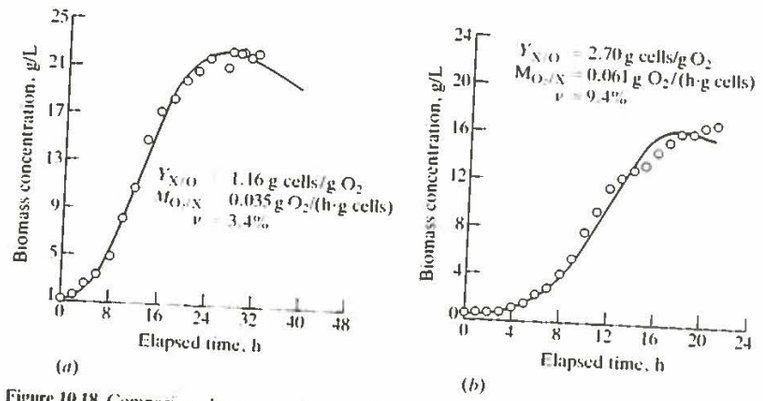

Figure 10.18 Comparison between measured (*dots*) and estimated (*line*) biomass concentrations for batch growth of (*a*) *Streptomyces* sp. and (*b*) *Saccharomyces cerevisiae*. (*Reprinted by permission from D. W. Zabriskie and A. E. Humphrey, "Real-Time Estimation of Aerobic Batch Fermentation Biomass Concentration by Component Balancing," AIChE J., vol. 24, p. 138, 1978.*)

Zabriskie and Humphrey [36] applied this approach to batch cultivation of several microorganisms. Figure 10.18 shows the results for (a) a *Streptomyces* fermentation and (b) for growth of *Saccharomyces cerevisiae*. Yield factor and maintenance coefficient values for each case are shown, as is the coefficient of variation r between experimental data (dots) and biomass concentration estimates (continuous line) based on Eq. (10.8). We see that this estimation procedure performs well for the *Streptomyces* cultivation but that, in the *S. cerevisiae* experiment, the data deviate qualitatively from the estimates. There is evidence of diauxic growth in the measurements which is not indicated by the estimates derived from material balancing. These discrepancies have been attributed to variations in $M_{O_2/X}$ and Y_{X/O_2} which accompany shifts in glucose utilization metabolism during cultivation [36].

This particular example is illustrative of an entire class of data analysis approaches based on macroscopic balances. These methods often perform well as gateways from measurements to derived process variable values. Difficulties may arise, however, because of error accumulation.

General methods for estimating the state of a process, that is the values of the variables which appear in a differential equation mathematical model of the process, have been developed by systems engineers and applied mathematicians. Like the material balancing approaches, these more advanced estimation methods take into account known relationships among process variables. However, these methods also consider noise effects and error propagation in fact, the mathematical bases for modern estimators rests in the theory of stochastic processes.

Presentation of modern multivariable *estimators* such as the *extended Kalman filter* is outside the scope of this text. We should, however, note several

attributes of the extended Kalman filter applied to bioreactors as revealed in the computational and experimental studies of San and Stephanopoulos [37, 38]:

1. The estimator responds rapidly and accurately to changes in process parameters.
2. The estimator is not sensitive to errors in the starting estimates of the state variables.
3. The state estimation method can be extended in a straightforward fashion to provide estimates of time-varying process parameters.
4. Error propagation and growth is not a problem with this type of estimator.

Figure 10.19 shows one computational test of the extended Kalman filter estimation method proposed by San and Stephanopoulos [31]. Here, a mathematical model of a chemostat was subjected to a series of step increases in dilution rate, and the model variables were corrupted by computer-generated noise before entering the estimator. The resulting changes in biomass density and specific growth rate are tracked accurately, even after the dilution rate has exceeded μ_{max} and washout has started to occur.

Process measurements and process variables and parameters deduced from the measurement data provide the information needed to operate the process. In

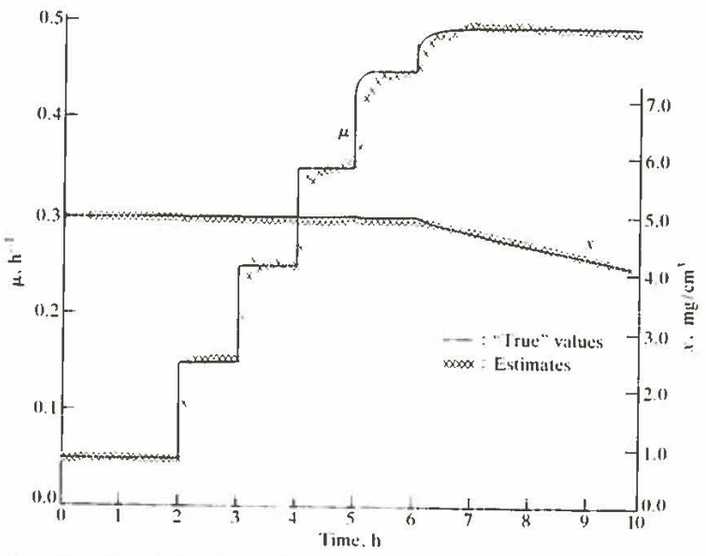

Figure 10.19 Comparison of simulated (*solid line*) microbial CSTR reponse to several successive shifts up in dilution rate compared to estimated values (*crosses*) obtained using an extended Kalman filter. (*Reprinted by permission from K. Y. San and G. Stephanopoulos, "Studies of On-Line Bioreactor Identification II. Numerical and Experimental Results," Biotech. Bioeng., vol. 26, p. 1189, 1984.*)

the next sections, we examine different strategies for maintaining and manipulating process operating conditions for good performance.

10.6 PROCESS CONTROL

Obtaining satisfactory process performance requires maintenance of operating conditions at design values. Because of unpredictable upsets which invariably enter a process due to fluctuations in pumping rates, flow patterns, mixer speed and other operating conditions, and because of chemical changes within the process, control action is usually required to maintain specified conditions. Sometimes we can improve batch reactor performance by varying process conditions such as pH or temperature in a predetermined fashion as the reaction occurs. Here, too, controls are required in order to carry out the desired batch operation program. In this section we first examine controls to maintain desired values of measured variables. Then, we consider control based on estimated process conditions.

10.6.1 Direct Regulatory Control

Using control of a bioreactor as an example, we frequently wish to control pH, temperature, aeration rate, agitation speed, and perhaps dissolved oxygen partial pressure at specified values. Since all these quantities can be measured on-line, regulation of each of these process state variables can be accomplished using a conventional *feedback controller*, the basic components of which are summarized in Fig. 10.20. Here, the controlled or output variable, say pH, is measured, and the analyzer output is sent to a controller where the measured output value is compared to the desired, or set point, value. Based on the deviation between desired and measured value, control action is determined by some control algorithm.

The controller may be a person who monitors instrument readout and decides what to do. More often, the controller is a pneumatic, electronic, or digital

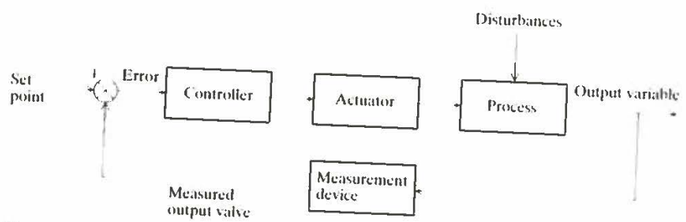

Figure 10.20 Elements in a feedback control system.

computer device. The simplest type of control is on-off control. Here, the actuator is turned on when the error exceeds a specified value and turned off when the error falls below another specified, threshold level, or vice versa. Such controls are used when the activator or control element, which acts physically on the process to cause corrective action, is an on-off device such as a single-speed pump. Thus, in on-off pH control, a pump which feeds base to the fermentor is turned on when the pH falls a certain amount (typically 0.25 pH units) below the set point pH. When the pH reaches a certain level (set point + 0.25 pH units) above the set point, the pump is turned off. (At this point, an acid feed pump may be turned on. However, this is usually not necessary since the effect of most fermentations is to lower medium pH.) Temperature control is often accomplished in a similar way, at least in small-scale reactors.

If the control element provides a continuous range of outputs, such as a variable-speed motor on the fermentor impellor shaft or a continuously adjustable valve on feed air supply, it is common to use *proportional-integral-derivative* (PID) control or some variation of this algorithm. For PID control, the controller output o is given by

$$o(t) \quad o_s + K_c\left[e(t) + \frac{1}{\tau_I}\int_0^t e(w)\,dw + \tau_D\frac{de(t)}{dt}\right] \tag{10.10}$$

Here, o_s is the nominal controller output corresponding to operation at the undisturbed, design condition, and $e(t)$ denotes the error

$$e(t) \quad \text{(set point value} \quad \text{measured value) at time } t \tag{10.11}$$

As indicated in Eq. (10.10), the three different terms in the bracket contribute to control action in proportion to the error (P), the integral of the error (I) and the derivative of the error (D). Relative weighting between these three control modes is determined by the parameters τ_I and τ_D which are called the *integral time* and the *derivative time*, respectively. The overall "strength" of control action is determined by the magnitude of the *proportional gain* K_c. If the derivative mode is absent ($\tau_D = 0$), the controller is called simply proportional-integral (PI). Other possibilities are obvious.

A properly adjusted controller of this type often provides excellent regulation of the measured variable. Poorly adjusted, a feedback controller can destablize the system, causing undesirable, accentuated fluctuations. How to set or "tune" a PID controller and its relatives is a central theme of many texts on process control (see, for example, Refs. 1–3).

Common practice is simultaneous use of several controllers of this type, one using temperature measurements to change cooling rate, one regulating pH, and so forth. Because of decreasing costs of digital computer hardware, it is increasingly effective to use a single microcomputer as the controller for several such single-variable feedback control loops. If the computer output (usually after a D/A converter or a relay) is used directly to drive the actuator, the system is said to be in *direct digital control* (DDC).

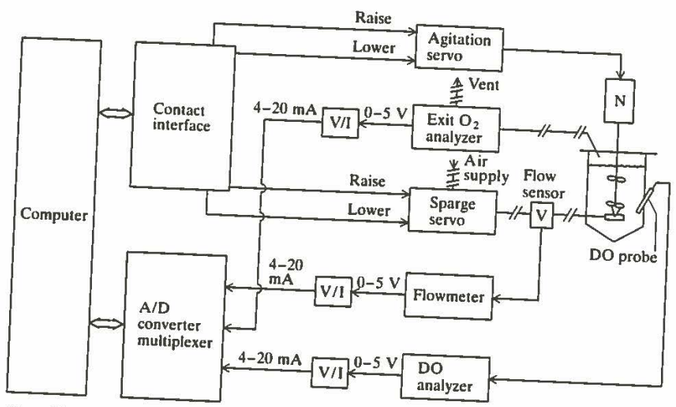

Figure 10.21 A computer control system for dissolved oxygen concentration based on exhaust gas and dissolved oxygen analyses. *(Reprinted by permission from L. P. Tannen and L. K. Nyiri, "Instrumentation of Fermentation Systems," p. 331 in "Microbial Technology," 2d ed., Vol. II [H. J. Peppler and D. Perlman (eds.)], Academic Press, New York, 1979.)*

A distinct advantage of using a computer as a controller is the opportunity to combine the data analysis capabilities of the computer with the flexibility of manipulating more than one process input to achieve control. An example of such a system is shown schematically in Fig. 10.21. Here, measurements of dissolved oxygen (DO) level and exit gas O_2 concentration allow on-line estimation of $k_l a$. This information can then be used in concert with the DO measurement to manipulate agitation rate and/or gas feed rate to control DO at the desired level. This is a DDC system. Notice that the A/D converter accepts current signals here, requiring conversion of instrument output voltages to currents (the V/I converter). It is preferable to transmit electrical analog signals in current form unless the transmission lines are very short (e.g., in a research laboratory) in order to avoid significant line losses. The theme of applying calculated process states and parameters for control is extended in the next section.

10.6.2 Cascade Control of Metabolism

The ultimate objective of any cellular bioreactor control scheme is to provide an environment or an environment history which drives the metabolic controls in the organism to maximize production of the desired compounds. Accordingly, instead of thinking about keeping pH or temperature at some particular value, it may be more useful to consider controlling the bioreactor so that the culture

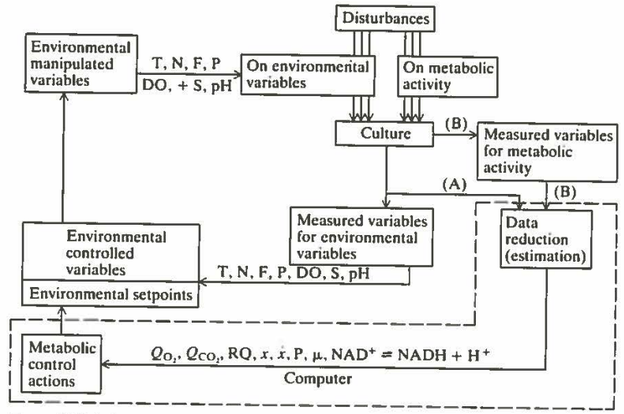

Figure 10.22 Information flow in a cascade control scheme in which deviations from desired metabolic states are used to modify environmental variables. *Legend:* DO, dissolved oxygen; F, gas flow rate; S, substrate; +S, substrate addition rate; P, pressure or product; N, agitation speed; Q_{O_2}, Q_{CO_2}, gas utilization rate. *(Reprinted by permission from L. P. Tannen and L. K. Nyiri, "Instrumentation of Fermentation Systems," p. 331 in "Microbial Technology," 2d ed., Vol. II [H. J. Peppler and D. Perlman (eds.)], Academic Press, New York, 1979.)*

growth rate or respiratory quotient is kept at a desired value. This is feasible since, as summarized in Sec. 10.5, we can estimate some metabolic properties of the culture based upon available measurements. Then, as illustrated in the information flowchart in Fig. 10.22, the estimated metabolic property may be compared with its set point value. Error here determines the "metabolic control action" in Fig. 10.22, perhaps using one of the feedback controller algorithms just described.

The output of the metabolic controller may be used directly to alter a process input such as a pumping rate. Alternatively, the metabolic controller output may be used to change the set point of an "environmental controller," say for pH or DO. The pH or DO controller will then alter the pH or DO which, in a good control system, will change the metabolic variable to reduce its deviation from the metabolic variable set point.

When the "environmental" variables are controlled by local single-loop controllers and the environmental controller set points come from a digital computer, the scheme is called *supervisory control* or *digital set point control* (DSC). Of course, everything may be done by digital computer/controllers. We have already seen such a system in Fig. 10.14, in which the first-level computer carries out the regulatory control of environmental variables, driven by set points provided by a higher level computer.

Computer control of fed-batch cultivation of baker's yeast (*Saccharomyces cerevisiae*) affords an interesting example of metabolic manipulation by proper environmental regulation. Regulation of glucose catabolism in this organism is quite complicated. At suitable values of glucose and dissolved oxygen concentration, glucose is utilized for respiration, providing maximal cell yields per unit amount of glucose consumed (recall Sec. 5.3). If glucose concentration increases above a certain level, metabolism switches to fermentation even in the presence of oxygen. This condition is termed *aerobic fermentation* and is the result of metabolic regulation known as the *Crabtree effect*. If aerobic fermentation occurs, cell yield on glucose is reduced, and ethanol and CO_2 are formed as end-products. As noted earlier (Sec. 7.2.3), ethanol inhibits yeast cell growth.

Consequently, aerobic fermentation should be avoided if production of yeast is the process objective (as is often the case). This can be accomplished by feeding glucose during the batch fermentation. The program of glucose feeding can be a preset schedule based on previous experience with the fermentation. However, due to batch-to-batch variability in the inoculum and medium (in practice, molasses rather than pure glucose), such a fixed feeding schedule may not match the glucose requirements of the culture over time, resulting in high glucose concentrations, aerobic fermentation, and yield reductions.

In order to adapt such a *feed-on-demand* strategy to the requirements for a particular batch, Wang, Cooney, and Wang [39, 40] used on-line material balancing to estimate the progress of the fermentation and to adjust the glucose feed rate accordingly. Respiratory quotient [RQ; Eq. (5.51)] was found to be a useful indicator of glucose utilization pathway, with RQ values greater than unity indicative of ethanol formation. RQ values in the ranges below 0.6, 0.7 0.8 and 0.9 1.0 signal ethanol utilization, endogeneous metabolism and oxidative growth, respectively.

Wang et al. [39, 40] described the baker's yeast fermentation using the following stoichiometric representation:

$$aC_6H_{12}O_6 + bO_2 + cNH_3 \rightarrow C_6H_{10.9}N_{1.03}O_{3.06} + eH_2O + fCO_2 \quad (10.12)$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2. \quad (10.13)$$

The empirical cell formula [with unity coefficient in Eq. (10.12)] is based on an average of elemental analyses conducted at different stages of the batch fermentation. There are seven unknowns in describing this reaction system: the five stoichiometric coefficients in Eq. (10.12), and the extents to which these two reactions have occurred. Based upon elemental balances on C, H, O, and N in Eq. (10.12) and measurements of O_2 utilization, CO_2 evolution, and NH_3 addition, these unknowns may be determined on-line.

Controlling glucose addition based on the strategy of maintaining RQ less than unity produced the results shown in Fig. 10.23. Here, the control and associated estimation method performed extremely well. Glucose and ethanol levels both remain low throughout the fermentation, with the exception of a brief pulse

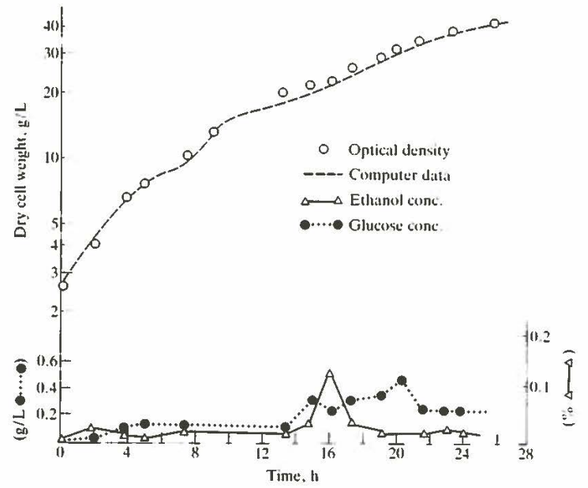

Figure 10.23 Results of a fed-batch baker's yeast fermentation using computer-controlled glucose feeding based on attaining desired respiratory quotient values. Measured and estimated biomass concentrations during the batch are shown for comparison. *(Reprinted by permission from H. Y. Wang, C. L. Cooney, and D. I. C. Wang, "Computer-Aided Baker's Yeast Fermentations," Biotech. Bioeng., vol. 19, p. 69, 1977.)*

in ethanol production around hour 16. The cell density trajectory obtained from material balancing computer estimates closely tracks the experimentally measured information.

Such close agreement between estimated and measured biomass concentrations was not obtained in other fed-batch experiments in which greater ethanol production occurred. In these cases, the cells consumed ethanol for growth, a reaction not considered in the biomass estimation procedure. These examples illustrate possible pitfalls in such direct material balancing approaches: errors in process state estimates, once made, tend to propagate, and existence of overall conversions not in the presumed stoichiometry can throw off the whole scheme.

10.7 ADVANCED CONTROL STRATEGIES

To conclude our overview of process instrumentation and control, we shall examine some of the strategies used to maximize product yield in batch reactors and to regulate and stabilize continuous reactors. In addition, we shall examine briefly some of the interesting scheduling and design problems which arise in a

process consisting of a sequence of different batch operations. In all of these cases, application of computers is essential to implement the control or to do the calculations necessary to determine the desired control strategy.

10.7.1 Programmed Batch Bioreaction

Given a particular organism, maximizing production from batch bioreactions requires determination of the environmental conditions during the batch which drive the cells to their best possible performance—or, stated differently—which maximize the genetic potential of the organism. There are several different ways in which this environmental optimization problem can be defined, depending on the instrumentation and control provided on the reactor. In the simplest case, exemplified by a shake flask in which there is usually no on-line measurement or control capability beyond operating temperature, we seek the best temperature and initial medium composition. In a bioreactor with only direct environmental controls, we can look for the pH, agitation intensity, and other environmental parameters which optimize performance. Here, the environmental variables are maintained constant throughout the batch reaction—to the extent feasible for the reactor. (For example, dissolved oxygen level will not be controllable if the culture oxygen demand exceeds the oxygen transfer capacity of the bioreactor.)

However, we know in many cases that a constant environment is not optimal for many fermentations. For example, secondary metabolites are not actively synthesized during rapid growth, but, on the other hand, slow growth is undesirable during the initial stages of a batch fermentation in which cell density is low. Accordingly, we should operate the reactor initially under conditions which maximize growth. Later, when cell density is high, we utilize conditions which stimulate maximum net product formation. In this production stage of the batch, consideration of the rate of product inactivation or decomposition as well as the rate of product synthesis is essential.

In a similar fashion, when manufacturing a protein using a recombinant cell, it is usually best to avoid expression of the product early in the batch because this often inhibits cell growth and may accentuate any genetic instability problems which exist. By adding an inducer (or depleting an inhibitor of gene expression) later in the batch, product formation can be switched on after suitable culture growth has occurred. Thus, operating strategies for recombinant batch fermentations may closely resemble those for secondary metabolites although the biochemical processes involved and their regulation are much different.

The activity of a batch culture at any point in time is a function of both the environmental state at that time and the previous history of culture environments. A particular time sequence of pH, dissolved oxygen level, and other variables may be required in order to develop the culture over time in a way that provides the greatest productivity. Often this is accomplished empirically. However, a sufficiently structured process model makes possible production maximization through computer simulation and optimization. In the remainder of this section we shall examine different examples showing the benefits of programmed

batch operation and the methods used to determine the batch programming strategy.

Production of the enzyme β-galactosidase by the mold *Aspergillus niger* in batch culture follows the pattern typical of secondary metabolites. A computer-controlled operating strategy for this process described by Lundell [41] is based upon the following guidelines:

Growth phase

1. Use carbon source feeding to extend the growth phase and increase the cell mass concentration.
2. Add additional carbon source intermittently as required, as determined by decrease of both the CO_2 evolution rate (CER) and respiratory quotient (RQ) to 20 percent of their maximum values.
3. Use the pH and temperature which maximizes cell growth.
4. To conserve energy, use the lowest possible air-feed rate and agitator rotation speed, as determined by the fermentor system's oxygen transfer rate capacity and the culture CER and RQ.

Enzyme formation phase

1. Switch to enzyme formation operating conditions when growth slows (dropping CER and RQ).
2. Use optimum temperature and pH for enzyme production.
3. Adjust agitator rotation and feed air-flow to meet process requirements (enzyme formation phase is characterized by lower oxygen requirement and decreased broth viscosity).
4. When enzyme formation rate slows, add more enzyme inducer.
5. Add additional nutrient to extend growth.
6. Add surfactant, then terminate the batch when enzyme formation rate declines rapidly.

Table 10.4 summarizes the different types of operating strategies which were examined experimentally. The continued batch (CB) mode employs nutrient feed during the enzyme formation phase. In the fed-batch (FB) mode, extra nutrient feed is provided during the growth phase. The FB/CB strategy combines both of these features. The enzyme production trajectories obtained using the conventional batch and continued batch operating actions are illustrated in Fig. 10.24. Performance characteristics of all four operating strategies are listed in Table 10.5. Here, careful selection of programmed batch conditions combined with computerized data analysis and control gives a 70 percent increase in enzyme production and simultaneously a 50 percent reduction in energy use. Notice that, although the basic features of the operating sequence are programmed in advance, on-line measurements and derived quantities are used to determine the particular points of switching from one operating region to another. This is very important in practice because of batch-to-batch variations in inocula and media.

It has been known for some time that high temperatures (30°C) maximize growth rate of the *Penicillium* mold while lower temperatures (20°C) are more favorable for high rates of penicillin synthesis. Although operation at a temperature between these extremes (24 to 25°C) has predominated in past commercial practice, intentional variation of the temperature during the batch, i.e., temperature programming, can conceivably give larger pencillin yields than any constant temperature. Standard mathematical procedures for obtaining the best temperature program have been applied to this problem [42, 43] with interesting results which are summarized next.

In order to apply optimal control theory, a mathematical model is necessary. The crosses in Fig. 10.25 are experimental data from commercial fermentors operated at 25°C. To avoid revelation of proprietary-process characteristics, these data were reported only in the nondimensional form shown. These plots do not show the lag phase: the first data point is about 50 h into the fermentation. The trends in these data suggest the following general forms of growth and product-formation kinetics:

$$\frac{dx_1}{dt} = b_1 x_1 \left(1 - \frac{x_1}{b_2}\right) \tag{10.14}$$

$$\frac{dx_2}{dt} = b_3 x_1 - b_4 x_2 + b_5 \frac{dx_1}{dt} \tag{10.15}$$

Table 10.4 Summary of different programmed batch operating strategies considered for optimization of β-galactosidase production by a strain of *Aspergillus niger*. In each case the total pressure was 130 kPa and the dissolved oxygen level was 20% of saturation

(Reprinted by permission from R. Lundell, "Practical Implementation of Basic Computer Control Strategies for Enzyme Production," p. 181 in "Computer Applications in Fermentation Technology," Society of Chemical Industry, London, 1982.)

1. Batch (B) fermentations used as reference.
   - temperature 30 °C
   - pH 4.5
   - stirring speed 200 rpm
   - air flow rate 30 m^3/h
2. Continued-batch (CB) fermentations with addition of extra organic nutrient to yield extended enzyme formation.
   - temperature 30 °C
   - pH 4.5
   - stirring speed 175 rpm/200 rpm/150 rpm
   - air flow rate 15 m^3/h 30 m^3/h 20 m^3/h
   - addition of extra organic nutrient
3. Fed-batch (FB) fermentation with addition of extra carbon source to provide extended cell formation.
   - temperature 35 °C/30 °C
   - pH 4.8/4.5
   - stirring speed 175 rpm/250 rpm/200 rpm
   - air flow rate 15 m^3/h 30 m^3/h 20 m^3/h
   - addition of starch and $(NH_4)_2HPO_4$
4. Fed-batch/continued-batch (FB/CB) fermentations with addition of extra organic nutrient during the enzyme formation phase.
   - temperature 35 °C/30 °C
   - pH 4.8/4.5
   - stirring speed 175 rpm/250 rpm/200 rpm
   - air flow rate 15 m^3/h 30 m^3/h 20 m^3/h
   - addition of starch and $(NH_4)_2HPO_4$ during the cell formation phase
   - addition of extra organic nutrient during the enzyme formation phase

where x_1 and x_2 are the dimensionless cell and penicillin concentrations, respectively. Equation (10.14) is the logistic equation familiar from Chap. 7, while (10.15) is of the Luedeking-Piret type, discussed before, with the addition of a term reflecting penicillin destruction. The need for this term is indicated by the flattening of the penicillin curve in Fig. 10.25 for large times. Also, other data indicate that penicillin hydrolyzes in aqueous solution and that the penicillin synthesis system may also decay with time.

Assuming various sets of values for parameters b_1 through b_8 and comparing the computed x_1 and x_2 time histories with the data show that the sum-of-squares residual for all data points is minimized at $T = 25$ °C when $b_8 = 0$, and

$$b_1 = 13.099 \quad b_{10} \quad b_2 = 0.9426 \quad b_{20}$$
$$b_3 = 4.6598 \quad b_{30} \quad b_4 = 4.4555 \quad b_{40} \qquad (10.16)$$

The temperature dependence of these parameters can be estimated by combining these values at 25 °C with (1) the known optimal temperatures for cell growth rate (30 °C) and penicillin synthesis rate

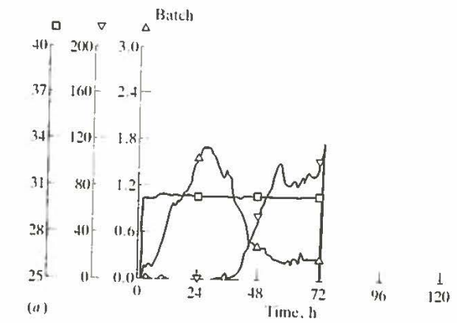

Figure 10.24 Data for β-galactosidase production by *Aspergillus niger* in (*a*) batch culture and (*b*, *c*) continued batch fermentation. The numbers in parts b and c designate computer-controlled changes in operating conditions during the batch as follows: 1, increase rotation speed; 2, decrease rotation speed; 3, constant rotation speed; 4, 5, 6, add organic nutrient; 7, add surfactant. *Legend*: □, temperature, °C; ▽, β-galactosidase activity; △, exhaust gas CO_2, %; ▼, ▲, fermentor weight, kg; ■, agitator rotation speed, s^{-1}; ○, respiratory quotient (kg/kg). *(Reprinted by permission from R. Lundell, "Practical Implementation of Basic Computer Control Strategies for Enzyme Production," p. 181 in "Computer Applications in Fermentation Technology," Society of Chemical Industry, London, 1982.)*

Table 10.5 Summary of the results of different batch fermentation operating strategies for maximizing production of β-galactosidase by *Aspergillus niger*
(Reprinted by permission from R. Lundell, "Practical Implementation of Basic Computer Control Strategies for Enzyme Production," p. 181 in "Computer Applications in Fermentation Technology," Society of Chemical Industry, London, 1982.)

Fermentation batch	Fermentation time, h	Enzyme formed, rel. act.	Energy used, kWh	Energy / enzyme, kWh / rel. act. / % of B	Enzyme / time, rel. act. h / % of B
B Conventional batch	72	100	180	1.8 / 100	1.39 / 100
CB Continued batch	115	207	210	1.0 / 56	1.80 / 129
FB Fed-batch	100	163	195	1.2 / 67	1.63 / 117
FB CB Fed-batch continued batch	120	283	240	0.85 / 47	2.36 / 170

(20 C) and (2) experimental evidence showing Arrhenius dependence of the rate constant for penicillin decay with an activation energy of 12 to 15 kcal mol. These facts suggest the forms

$$\begin{aligned} b_i(\theta) &= b_{i0}g(\theta) \qquad i = 1, 2 \\ g(\theta) &= 1.143[1 - 0.005(30 - \theta)^2] \\ b_3(\theta) &= 1.143b_{30}[1 - 0.005(\theta - 20)^2] \\ b_4(\theta) &= b_{40}\exp\left[6145\left(\frac{1}{273.1+\theta} - \frac{1}{298}\right)\right] \end{aligned} \tag{10.17}$$

where θ is temperature in degrees Celsius.

Utilizing all the above equations, we can now cast the fermentation model in the general form

$$\frac{dx_i(t)}{dt} = f_i(x_1(t), x_2(t), \theta(t)) \qquad i = 1, 2 \tag{10.18}$$

with initial values

$$x_1(0) = 0.0294 \qquad x_2(0) = 0 \tag{10.19}$$

The objective of maximizing penicillin yield can now be precisely stated mathematically. Find θ as a function of t from $t = 0$ to the final fermentation time $t = T$ such that $x_2(T)$ is maximized. The

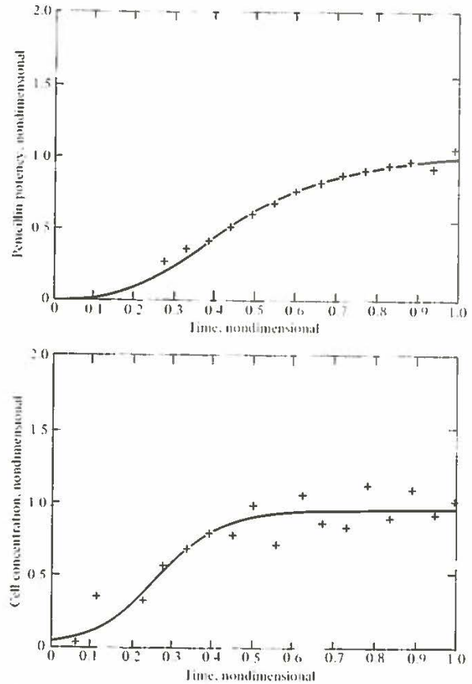

Figure 10.25 Experimental data for a commercial batch penicillin fermentation at 25 C. *(Reprinted by permission from A. Constantinides, J. L. Spencer, and E. L. Gaden, Jr., "Optimization of Batch Fermentation Processes. I. Development of Mathematical Models for Batch Penicillin Fermentations," Biotech. Bioeng., vol. 12, p. 803, 1970.)*

Maximum Principle [5] asserts that for the optimal temperature program, which we shall denote θ^*, it is necessary that the Hamiltonian function H

$$H(\theta) = \sum_{i=1}^{2} \lambda_i^*(t) f_i[x_1^*(t), x_2^*(t), \theta] \tag{10.20}$$

be a maximum for $\theta = \theta^*(t)$ for all t between 0 and T. The superscript * in Eq. (10.20) denotes values obtained using θ^*, where the adjoint variables λ_i satisfy the differential equations

$$\frac{d\lambda_i}{dt} = \sum_{j=1}^{2} \lambda_j(t) \frac{\partial f_j[x_1(t), x_2(t), \theta(t)]}{\partial x_i} \tag{10.21}$$

and the conditions

$$\lambda_1(T) = 0 \qquad \lambda_2(T) = 1 \tag{10.22}$$

These necessary conditions and associated theoretical results suggest the following iteration algorithm for computing the optimal temperature program:

1. Let $\theta^{(n)}(t)$ denote the nth guess for the program.
2. Solve Eqs. (10.14) and (10.15) for $x_1^{(n)}(t)$, $x_2^{(n)}(t)$ using $\theta = \theta^{(n)}$.
3. Using $x_1^{(n)}$, $x_2^{(n)}$, $\theta^{(n)}$, to evaluate the time-varying coefficients on the right-hand side of Eq. (10.21), integrate those equations backward numerically from $t = T$ to $t = 0$ to determine $\lambda_1^{(n)}(t)$, $\lambda_2^{(n)}(t)$ (this reverse integration is necessary to avoid numerical instabilities often encountered if this integration is begun at $t = 0$).
4. Compute

$$\frac{\partial H^{(n)}}{\partial \theta}(t) = \sum_{i=1}^{2} \lambda_i^{(n)}(t) \frac{\partial f_i(x_1^{(n)}(t), x_2^{(n)}(t), \theta^n(t))}{\partial \theta} \tag{10.23}$$

Determine the $(n+1)$st guess for the optimal program using

$$\theta^{(n+1)}(t) = \theta^{(n)}(t) + \varepsilon \frac{\partial H^{(n)}}{\partial \theta}(t) \tag{10.24}$$

where ε is a small positive number.

Constantinides, Spencer, and Gaden [42, 43] employed this procedure with some adaptations to avoid decreasing cell concentrations. The final results are illustrated in Fig. 10.26. Notice that the temperature is initially large to maximize cell growth and then lower to optimize penicillin formation rate. The increase in penicillin yield provided by optimal temperature programming is quite dramatic: the yield is 76.6 percent larger than that obtained at the best constant temperature of 25 C.

Other models based on different data sets are formulated and optimized in the references mentioned earlier. In those cases, penicillin-yield improvements of about 15 percent result from programmed temperature. The temperature variations prescribed by these calculations can be approximated closely in commercial practice with little added cost. Consequently, the combined tools of mathematical modeling and optimization theory should also find fruitful application for other fermentations.

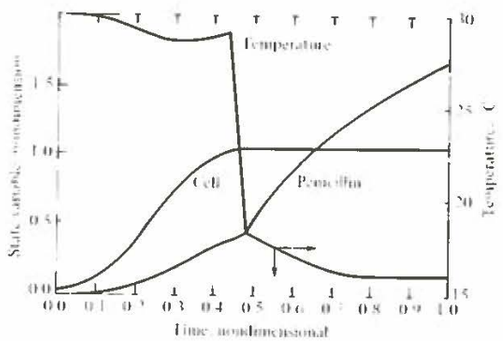

Figure 10.26 Computed optimal temperature policy and the corresponding growth and penicillin-production profiles. (*Reprinted by permission from A. Constantinides, J. L. Spencer, and E. L. Gaden, Jr., "Optimization of Batch Fermentation Processes. II. Optimum Temperature Profiles for Batch Penicillin Fermentations," Biotech. Bioeng., vol. 12, p. 1081, 1970.*)

10.7.2 Design and Operating Strategies for Batch Plants

An overall manufacturing process consisting of a series of different types of batch operations (e.g., substrate pretreatment, sterilization, fermentation, product recovery, packaging) poses a hierarchical series of problems from single units to overall process-to-process design and scheduling for manufacture of several different products. These may be summarized as follows [44]:

Characterize and optimize performance of individual process units.
Optimize the performance in a given sequence of batch process units producing a single product.
Specify equipment required for manufacture of one or several products.
Determine equipment interconnections to meet product requirements most efficiently.
Decide the operating strategies to be used in manufacturing several different products over a certain time span.

We have already considered the first of these problems in several contexts. To see how consideration of a sequence of batch processes can alter optimum design, we shall consider a simple example in which a function $f(t)$ describes the fraction of feed raw material converted during operating time t to useful products for the next stage. In addition, we assume the time to clean the process unit and charge it for the next batch is t_{cl}. The objective function F considered here is the amount of feed converted per unit time which may be written

$$F(t) = \frac{f(t - t_{cl})}{t} \qquad t \geq t_{cl} \tag{10.25}$$

Differentiating Eq. (10.25), equating the result to zero and rearranging this condition on the optimum cycle time (denoted t^*) gives the relationship

$$f'(t^* - t_{cl}) = \frac{f(t^* - t_{cl})}{t^*} \tag{10.26}$$

As sketched in Fig. 10.27, this condition has a simple graphical interpretation: the optimum batch cycle time is the point at which a straight line through the origin is tangent to the unit's performance function $f(t - t_{cl})$. This construction is shown also for a second batch process with performance function g.

Now consider two batch processes in series, the first characterized by f and the second by g. The fraction of raw material converted to desired product in the serial batch process is $f(t - t_{cl})g(t - t'_{cl})$ which we will call $h(t)$. Here, t'_{cl} is the restart time for the second unit. Maximizing feed conversion per unit time requires maximization of

$$F = \frac{f(t - t_{cl})g(t - t'_{cl})}{t} = \frac{h(t)}{t} \qquad t \geq \max\{t_{cl}, t'_{cl}\} \tag{10.27}$$

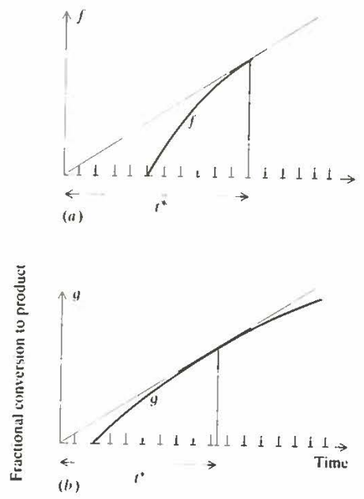

Figure 10.27 Performance curves and determination of optimum cycle times for single-batch units (*a*, *b*) and for two batch units in series (*c*).

Considering the same calculations as before, we arrive at the same condition for tangency, but now in terms of the composite performance function h (Fig. 10.27*c*). The following central point is illustrated by the example performance curves and graphical constructions in Fig. 10.27: the optimum cycle time for batch processes in series may be different from the optimum cycle time for any of the individual processes treated separately.

If the optimum cycle times for individual steps in a batch-processing sequence are significantly different, improved overall performance may be possible by allowing different cycle times for different units. The simplest case which allows different cycle times is selection of a cycle time for the slowest unit(s)

which is an integer multiple (m) of the faster unit(s) cycle times. Then m of the slower units must be provided in parallel to keep up with process transitions from and to the faster unit(s).

Maximum flexibility in cycle time selection for batch-unit processes in series is afforded by including *intermediate storage* in the plant, so that products from one batch unit can be stored temporarily before entering the next batch unit in the processing sequence. Intermediate storage can also provide other useful functions in batch processing [45]. Just as storage or surge tanks in continuous processes can absorb transients, intermediate storage in batch processes helps to damp disturbances created by equipment failures, by fluctuations in unit start-up time, and by batch-to-batch variation in operating performance as is common, for example, in batch fermentation. Also, intermediate storage serves to moderate upsets which may arise in switchover from one product to another.

Several requirements must be kept in mind when intermediate storage is considered. First, enough units of each type must be provided in parallel so that the time-average processing capacity is equal for all steps in the overall process. Second, the stability of the raw materials and products under storage conditions must be considered. Intermediate storage is impractical for unstable materials. Finally, requirements for *batch integrity* may preempt any economic advantages for certain batch-process operating strategies. Batch integrity means that the material from one batch is processed separately from any other batch's material —with no intermixing at any point. In this way, a certain lot of product can be identified uniquely with a corresponding batch of production. This requirement is common in the pharmaceutical industry. Intermediate storage may still be desirable to address cycle time imbalances or to improve damping functions even when batch integrity must be maintained.

Many fascinating design and optimization problems and opportunities arise in consideration of equipment specification and in design and operation of multiproduct plants. Recent research on these and other aspects of batch (or semicontinuous) process design are reviewed in the chapter references.

10.7.3 Continuous Process Control

Different types of control problems and opportunities for use of advanced control strategies arise for continuous processes. Here, the system is usually designed for operation at some steady-state condition. One control objective in this case is minimizing start-up time or some function of start-up costs. This is essentially a batch-optimization problem of a somewhat different type from those just considered — now the goal is to move the system from its initial state to the desired operating point or some neighborhood of that point in order to minimize some objective function. Given a good process model, this problem can be solved by methods related closely to that described above for batch temperature programming for penicillin production.

After start-up, the usual control objective is keeping the process at the desired steady state. This can often be accomplished using several separate,

single-loop controllers of the kind discussed in Sec. 10.6, and indeed this is current standard practice in biochemical processing. However, experience in system and process control in other contexts has shown that undesirable *interactions* often occur between different control loops. That is, when manipulating one process input to try to drive one process variable back toward its set-point value, other process variables are disturbed. Generally, many process variables are coupled with each other and with several process inputs, causing individual control loops to interact.

Several different approaches have been developed for dealing with control interactions. Most of these we must leave to the chapter references, but one, optimal multivariable control, will be considered briefly here.

First, we recall that a process system described by

$$\frac{d\mathbf{c}(t)}{dt} = \mathbf{f}[\mathbf{c}(t), \mathbf{d}(t)] \tag{10.28}$$

where $\mathbf{c}$ is the vector of process state variables and $\mathbf{d}$ is a vector of process inputs, can be approximated near a steady-state operating point ($\mathbf{c}_s$, $\mathbf{d}_s$) satisfying

$$\mathbf{f}(\mathbf{c}_s, \mathbf{d}_s) = \mathbf{0} \tag{10.29}$$

by the linearized differential equation

$$\frac{d\boldsymbol{\chi}(t)}{dt} = \mathbf{A}\boldsymbol{\chi}(t) + \mathbf{B}\mathbf{v}(t) \tag{10.30}$$

where $\boldsymbol{\chi}$ and $\mathbf{v}$ are the state and input deviations, respectively (see Sec. 9.2):

$$\boldsymbol{\chi}(t) = \mathbf{c}(t) - \mathbf{c}_s \tag{10.31}$$

$$\mathbf{v}(t) = \mathbf{d}(t) - \mathbf{d}_s \tag{10.32}$$

We base the multivariable control design on the local, linearized approximation of Eq. (10.30).

To formulate a mathematical optimization problem compatible with the goal of keeping $\mathbf{c}(t)$ near $\mathbf{c}_s$, or $\boldsymbol{\chi}(t)$ near zero, we shall seek to minimize the scalar objective function

$$J = \tfrac{1}{2}\int_0^\infty [\boldsymbol{\chi}^T(t)\mathbf{C}\boldsymbol{\chi}(t) + \mathbf{v}^T(t)\mathbf{R}\mathbf{v}(t)]\, dt \tag{10.33}$$

where $\mathbf{C}$ and $\mathbf{R}$ are positive definite matrices. The first term in the integrand in Eq. (10.33) has clear physical significance: this is the measure in some sense of the amount of deviation of the state from the set-point vector $\mathbf{c}_s$, and the integral adds together all of the instantaneous values of this measure of off-spec operation. How $\mathbf{C}$ is chosen is up to the control designer, who may wish to weight some variable deviations more than others based on the requirements or economics of that particular process. The simplest choice of $\mathbf{C}$ is the identity matrix, which makes the first term in the integrand simply the sum of the squares of all the state variable deviations.

The term $\mathbf{v}^T\mathbf{R}\mathbf{v}$ in the objective function is sometimes called the cost of control, but this interpretation usually does not make physical sense in the context of chemical process control. This term serves an essential mathematical and practical function: without it, the optimal control would be unbounded and thereby physically impossible. It is more useful to think of $\mathbf{R}$ as a matrix which scales the magnitude of the control action, with larger values of the norm of $\mathbf{R}$ corresponding to smaller deviations of the process inputs from their nominal values under steady-state design conditions.

Applying the Maximum Principle introduced in Sec. 10.7.1, we can show that the control which minimizes J in Eq. (10.33) subject to the state and control interactions described by Eq. (10.30) (often called the linear-quadratic optimal control problem) is given by

$$\mathbf{v}(t) = -\mathbf{R}^{-1}\mathbf{B}^T\mathbf{M}\boldsymbol{\chi}(t) \tag{10.34}$$

where the time-invariant matrix $\mathbf{M}$ satisfies the following nonlinear algebraic equation:

$$\mathbf{MA} + \mathbf{A}^T\mathbf{M} + \mathbf{C} - \mathbf{MBR}^{-1}\mathbf{B}^T\mathbf{M} = \mathbf{0} \tag{10.35}$$

This is an interesting result, since Eq. (10.34) has the form of a multivariable, feedback control. Thus, current control values are given explicitly in terms of the current values of the state variables.

If the state variable cannot be measured directly, we may be able to estimate these values based on one of the data analysis methods described earlier. Kalman filter methods are especially suitable here since filter calculations are formulated in a mathematical framework very similar to that used in calculating the optimal multivariable feedback control. Comparisons of controller performance with direct state measurements and results based on Kalman filter estimates for a simulated fermentation process are described in Ref. 46.

Fan, Erickson and coworkers [47] studied using mathematical models the response of a single biological CSTR in which cell growth was described by Monod kinetics with maintenance. An optimal controller of the design just described was used to manipulate flow rate through the vessel based upon measurements of effluent substrate and cell mass concentrations. The control design matrices $\mathbf{Q}$ and $\mathbf{R}$ were taken as

$$\mathbf{Q} = \begin{pmatrix} q_{11} & 0 \\ 0 & q_{22} \end{pmatrix} \qquad \mathbf{R} = 1 \text{ (scalar)} \tag{10.36}$$

With $\mathbf{R}$ fixed, increasing one or both nonzero components of $\mathbf{Q}$ has the effect of increasing the amount of control action.

Shown in Fig. 10.28 are the variations in volumetric flow rate (the manipulated input or control variable) and corresponding trajectories in dimensionless effluent cell density and substrate concentration following a 12.5 percent step increase in feed concentration from dimensionless time zero to dimensionless time = 2. The parameters on these trajectories correspond to different magnitudes of q_{11} with q_{22} fixed at 1000. Notice that as q_{11} increases, the control variable changes more, the deviations in dimensionless effluent substrate concentration (y_1) decrease, while larger fluctuations are obtained in dimensionless biomass concentration (y_2). Keeping q_{11} fixed and increasing q_{22} gives different control action during the disturbance which reduces y_2 deviations and gives greater y_1 deviations for increasing control action. This shows the ability to tune the controller response and effects according to the process requirements.

Another possible goal of continuous process control is stabilizing a steady state that is unstable in the absence of control. Two examples can be cited from the recent research literature. Growth in a CSTR of methanol-utilizing organisms which exhibit substrate-inhibited kinetics admits, according to reactor models, three steady states for some operating conditions. DiBiasio, Lim and Weigand [48, 49] showed theoretically and experimentally that stable operation at the intermediate, unstable steady state could be obtained by use of proportional control. The measured process output was culture turbidity and the manipulated variable was substrate feed rate.

Although operation at a desired steady state is usually considered the ideal for continuous reactor operation, a number of computational and experimental

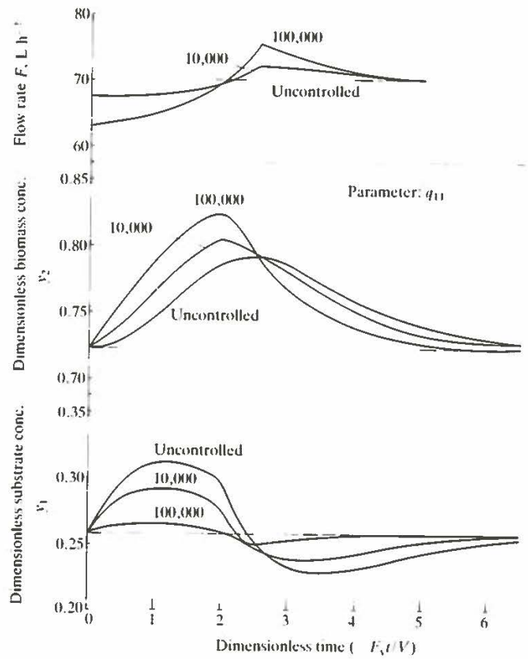

Figure 10.28 Simulation results showing application of an optimum linear-quadratic control to a microbial CSTR. The parameter on the different trajectories is q_{11}, and the dashed lines denote the reference steady-state values of dimensionless substrate concentration ($y_1 = s/s_0$) and dimensionless biomass concentration ($y_2 = x/Y_{x/s}s_0$). $q_{22} = 1000$ throughout. *(Reprinted by permission from L. T. Fan, P. S. Shah, N. C. Pereira, and L. E. Erickson, "Dynamic Analysis and Optimal Feedback Control Synthesis Applied to Biological Waste Treatment," Water Research, vol. 7, p. 1609, 1973.)*

studies indicate that, in some cases, improved reactor performance can be obtained in dynamic operation which is forced by time variations in feed or operating conditions [50]. Periodic operation, in which such forcing involves periodic functions of time and the reactor variables eventually become periodic also, is the most widely considered dynamic operating mode. The basic concept underlying the possibility of improved reactor operation by intentional transient operation is quite simple. In transient operation, medium and cellular compositions and reaction rates can achieve different interrelationships than any of those possible in

steady-state operation, where steady-state stoichiometric constraints limit the available mixture compositions. Intentional periodic fluctuations in bioreactor operation have been shown, for example, to increase cytochrome production by *Candida utilis*, biophotolytic hydrogen yield by *Anabaena cylindrica*, and to modify macromolecular composition of *E. coli* [51].

As we will explore further in Chap. 13, growth of two different organisms competing for a common limiting substrate usually leads to washout of one species. However, Hatch, Cadman and Wilder [52] have shown in simulation studies that a stable steady state with both species coexisting can be maintained using a proportional control algorithm in which substrate feed rate and overall dilution rate are both manipulated in response to measurements of the cell densities of the two species. Further, they demonstrated experimentally the feasibility of rapidly monitoring the cell concentrations of both *Candida utilis* and *Corynebacterium glutamicum* using flow cytometer light-scattering measurements.

10.8 CONCLUDING REMARKS

Control of a biotechnological process, whether by human or computer, requires good information on process operating state. This in turn depends upon analytical instrumentation and rigorous, systematic analysis and interpretation of process data. Major advances should occur in coming years as more powerful analytical tools from chemistry, biochemistry, and cell biology are adapted and improved for process applications. Model-based data analysis methods will progress beyond applications of elementary stoichiometry to on-line parameter updating and more detailed metabolic state estimates based on robust, structured models.

Several major concepts and topics connected with process control which were not discussed above should be mentioned before turning to our next topic, separation processes. On a local level, we wish to conduct the process according to some design or operating specification. That specification arises in turn from a higher level optimization or control problem in which the goal is maximization in some sense, often profit, of the contribution of that process to the overall plant, thence the parent corporation, and finally to the society. The objective functions and the constraints which the process engineer considers are derived from a hierarchy of objectives and rules at larger levels of operation.

Often reliability and safety are important components of the objective function or the operating constraints. How should the process be controlled to maximize product uniformity, to minimize the chances of losing a batch, and to minimize the probability of worker exposure to potentially unhealthful conditions? In practice, these objectives may be much more important than a few percent more or less in product yield or deviation from set point. Control engineers have begun to explore these questions, and we can expect more advances in these areas and in their significance in bioprocess control design in the future.

PROBLEMS

10.1 On-line sensors Define and give, in one sentence, the operating principle of the following.

(*a*) Thermistor, thermocouple, diaphragm gauge, Hall effect wattmeter, torsion dynamometer, strain gauge, gas rotameter, thermal mass flowmeter, capacitance probe.

(*b*) pH electrode, galvanic and polarographic types of dissolved oxygen probe, p_{CO_2} electrochemical probe, immobilized enzyme electrode.

(*c*) Mass spectrometer, gas chromatograph, paramagnetic O_2 analyzer.

(*d*) Spectrometer, nephelometer, in situ fluorometry.

10.2 Computer and control functions Define (*a*) A/D converter, multiplexer, D/A converter, (*b*) hardware, software, (*c*) process, sensor, monitor, controller, final control element.

10.3 State and parameter estimation (*a*) Discuss *measured* vs *estimated* variables. (*b*) What hardware and/or software system is needed for state estimation?

10.4 Process control and Laplace transforms The Laplace transform has properties very convenient for evaluation of linear, or linearized, control systems. The definition of the transform of any function of time, $f(t)$ is $F(s)$ where

$$F(s) \equiv \int_0^\infty f(t)e^{-st}\,dt$$

which is often abbreviated as $F(s) \equiv L\{f(t)\}$.

(*a*) Establish the properties of transforms of a derivative and integral:

$$L\{df/dt\} = sF(s) \qquad \text{if } f(t=0) = 0$$

$$L\left\{\int_0^t f(t)\,dt\right\} = F(s)/s$$

(*b*) Show that the Laplace transform of the controller output deviation $o(t) - o_s$ (Eq. 10.10) is

$$O(s) = K_c\left\{E(s) + \frac{E(s)}{\tau_I s} + \tau_D E(s)\right\}$$

and thus that the transfer function, or ratio of output (correction) to input (error) transforms, is given in the s-domain by

$$\frac{O(s)}{E(s)} = K_c\left[1 + \frac{1}{\tau_I s} + \tau_D s\right] = G_c(s)$$

10.5 pH control The kinetics of product formation during the batch lactic acid fermentation was described by Luedeking and Piret as Eq. (7.93)

$$\frac{dp}{dt} = \beta x + \alpha\frac{dx}{dt}$$

(*a*) Suppose $x(t)$ is described, under approximately constant pH, by the logistic equation

$$dx/dt = \mu x(1 - x/x_{max})$$

Obtain a solution for $p(t)$ assuming that $p(0) = 0$.

(*b*) Lactic acid ($pK_a = 3.88$) causes a pH shift if the fermentation is uncontrolled. Suppose we wish to control the pH at 6.5 (i.e., the desired "set-point"). Derive expressions for the error in an uncontrolled process

$$e(t) \equiv \text{pH}(t) - 6.5$$

What time program for addition of a small stream of concentrated base will keep the error in pH no larger than 0.1 ($\equiv \text{pH} - \text{pH}_0$)?

(*c*) Derive an expression for the output $O(t)$ of a PID controller using Eq. (10.10) and the error $e(t)$ of the uncontrolled process.

(*d*) Consider a proportional-only controller (Eq. 10.10 with $\tau_D = 0$ and $\tau_I = \infty$). The largest error allowable ($\text{pH} - \text{pH}_0 = 0.1$) must generate a base addition rate $\dot{m}_B$ which just matches the maximum product generation rate $V_R(dp/dt)_{max}$. Expressing the output $O(t)$ as equal to the base addition rate $\dot{m}_B$, what proportional gain K_c is needed for satisfactory control?

(*e*) For the simple block diagram shown in Fig. 10P5.1 derive an expression for pH(t) for the *controlled* process. Neglect the volume change caused by addition of concentrated base, $\dot{m}_B$. Assume that $\text{pH}_m = \text{pH}$, i.e., that the pH probe is both instantaneous and accurate. (For information on block diagram representations, see any process control text such as D. R. Coughanowr and L. B. Koppel, *Process Systems Analysis and Control*, McGraw-Hill, New York, 1965.)

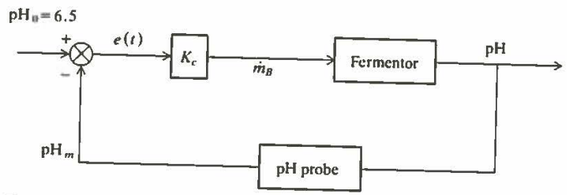

Fig. 10P5.1

10.6 Time lag in transient conditions In the presence of transients, the introduction of a time delay constant ($= 1/\gamma$) for the response of the instantaneous specific growth constant $\mu(t)$ to the changed substrate level has been proposed (T. B. Young, D. F. Bruley, and H. R. Bungay, "A Dynamic Mathematical Model of the Chemostat," *Biotect. Bioeng.*, **12**: 747, 1970). The movement of $\mu(t)$ toward the steady-state value μ_0 is given by

$$\mu_0 = \frac{\mu_{max}s}{K_s + s} \qquad \frac{d\mu}{dt} = \frac{\mu_0 - \mu}{\gamma}$$

(*a*) If s is a function of time $s(t)$, show that the solution is given by

$$\mu(t) = \mu(t=0)e^{-t/\gamma} + \frac{\mu_{max}}{\gamma}\int_0^t \frac{e^{-(t-t')/\gamma}s(t')\,dt'}{K_s + s(t')}$$

(*b*) Suppose $s(t) = s_0(1 + \alpha\cos wt)$, $\alpha < 1$, and $K_s \gg s(t')$. Obtain the explicit solution for $\mu(t)$ above, plot the ratio $\mu(t)/\mu_{max}$ vs. t for $\alpha = 0.5$, $\gamma = 1$, and $w = 0.1\gamma$, 1.0γ, and 10γ. Explain the differences between these curves. For what sorts of transients ought the time constant γ be included?

(*c*) If growth $r_x = \mu xs$, write down the equations needed to describe the behavior of a CSTR if $s_f = s_{f0}(1 + \cos wt)$.

10.7 Aerated chemostat monitoring When growing aerobic organisms in a chemostat, compressed air is passed through the fermentor at typically one volume of air per volume of liquid per minute (VVM). Compressed air is quite dry on entering the fermentor but saturated with water on leaving. As a consequence, the liquid flow rate in and out will be different.

How will this affect the interpretation of analytical data obtained by measurement on the fermentor effluent? If the fermentor volume is 10 liters, and the dilution rate is 0.1 h^{-1}, what will be the actual product concentration, corrected for evaporation, in the following system:

s_0 50 g glucose/liter

K_s 10 mg glucose/liter

μ_{max} 0.7 h^{-1}

$Y_{X/S}$ 0.5 g cell/g glucose

m 0.02 g glucose/g cell-h

$Y_{P/S}$ 0.5

q_P 0.2 g product/g cell-h

T 60 C

The vapor pressure of water at 60 C is 150 mmHg. Assume that Monod model is valid. Also you may assume that the concentration of CO_2 in the air is negligible and that the respiratory quotient for this fermentation is unity.

10.8 Feedforward control A batch fermentation is operated such that microbial growth is given by

$$\frac{dx}{dt} = \mu x$$

until a critical nutrient S is exhausted at $(x - x_0) = Y s_0$. The aerobic process has stoichiometric requirements for oxygen for biomass growth and for product formation, reflected in Y_{X/O_2} and Y_{P/O_2}.

(*a*) If product formation is nongrowth associated, so that $dp/dt = \beta x$, derive expressions for the instantaneous oxygen demand, Q_{O_2}, and for the specific oxygen demand (based on cell concentration), Q_{O_2}/x. At what condition do each of these have maximum values?

(*b*) Derive an expression for the $K_L a$ design value needed to meet the peak demand.

(*c*) If $K_L \sim$ constant and a varies as the gas inlet flow rate to the 3/4 power, how must F_g (inlet) be programmed to just achieve the necessary aeration rate with a minimum total gas utilization. Derive an expression for the ratio of air volume delivered, as cell mass grows from x_0 to x_{max}, divided by the volume delivered if F_g were held at the maximum value throughout this time.

(*d*) Feedforward control is often easier to implement than feedback control, but since the former involves no continued sensing of the process, it runs "blind." Discuss the consequences of having the programmed airflow meter for part (*c*) miscalibrated so that it consistently delivers (i) 10% below or (ii) 50% above the desired program rate. What modifications in the control system do you propose to address this potential problem?

10.9 State estimation via on-line mass balancing (*a*) Consider the rapid growth of cells during which the CO_2 production rate (CPR) is proportional to the biomass growth rate. Derive expressions for estimates of x and μ (i) if CPR is measured continuously and (ii) if CPR is measured at discrete intervals of length Δt.

(*b*) At low cell growth rates, corresponding to penicillin production, the simple CO_2 proportionality breaks down and a fuller carbon balance is needed. The overall reaction in a feed-batch system is given by

Substrate + penicillin precursor + seed biomass →
unconverted substrate + unconverted precursor + fermenter biomass
+ soluble unidentified products + penicillin + CO_2

If only substrate, precursor, CO_2, and penicillin provide appreciable carbon in addition to the biomass, express the biomass concentration through a carbon balance, in terms of measurable substrate, precursor, penicillin and CO_2 level, letting γ_i = fraction carbon in ith species. Ignore the volume change of fermentation fluid with time.

(*c*) Suppose that penicillin is not directly measurable, but that it does not contribute importantly to the carbon balance. If penicillin accumulation is assumed to have the kinetics given earlier, such that

$$\frac{dp}{dt} = nx + m\frac{dx}{dt} - kp$$

derive an expression from which you could estimate p concentration in terms of discrete measurements of substrate, precursor, and CO_2 levels. (see D.-G. Mou and C. L. Cooney, *Biotech. Bioeng.*, **25**, 225, 257, 1983, for a detailed example of on-line balancing for penicillin production).

10.10 Automatic supplementation of minerals in fed-batch culture *In situ* probes for NH_3 or volatile carbon substrate (e.g., ethanol) allow direct feedback control of feed addition rate of nitrogen or carbon. Many minerals necessary for growth and viability cannot be measured on-line. Full mineral addition at $t = 0$ would often be inhibitory to the inoculum. Accordingly, fed-batch programmed addition of minerals can be accomplished by use of stoichiometric relations between the measurable and nonmeasurable *in situ* levels.

Consider a simple balance of the following type:

$$\alpha(NH_3) + \beta(\text{carbon source}) + \gamma(\text{mineral source}) \rightarrow \text{biomass}$$

(*a*) For three feed reservoirs at concentrations n_0, c_0, and m_0 of nitrogen, carbon, and mineral, respectively, what ratio of volumetric feed rates will be stoichiometrically balanced for biomass production? How many different sensors are needed to set all feed rates?

(*b*) If both biomass and a product ($C_7NO_3H_{11}$) are produced, derive an expression for the proper volumetric mineral solution rate, $F_m(t)$, in terms of the feedback-controlled rates of nitrogen and carbon solutions, $F_n(t)$ and $F_c(t)$. How many different sensors are now needed? (See T. Suzuki et al. "Automatic supplementation of Minerals in Fed-Batch Culture to High Biomass Concentrations," *Biotech. Bioeng.*, **27**: 192, 1985.)

10.11 Enzyme assay for growth monitoring Studies on the microbial utilization of cellulose are difficult because of the water insolubility of the substrate. You are asked to examine the production of the enzyme glucose oxidase by a cellulolytic bacterium growing on cellulose. Glucose oxidase catalyses the reaction:

$$\text{Glucose} + O_2 \rightarrow \delta\text{-gluconolactone} + H_2O_2$$

Kinetics of this reaction may be measured with an enzyme-linked colorimetric assay or with a manometric apparatus (e.g. Warburg respirometer). You know that the organism of interest contains about one unit of glucose oxidase activity per gram of cell protein (1 unit = 1 μmole of glucose consumed/minute). The cellulase activity in this bacterium is associated with the cell wall, thus direct contact between the organism and substrate is required for growth.

(*a*) Design an experiment to study the kinetics of growth and glucose oxidase production by the bacterium growing on cellulose powder (particles are 100 μm in diameter). These studies should include both the exponential and stationary phases. In particular, you should describe: (1) the type of apparatus you would use, (2) the specific type of assays, and (3) the amount of cellulose and nitrogen source [$(NH_4)_2 SO_4$] to be added to the medium.

(*b*) How long would you expect the experiment to take and what would you expect the growth and enzyme specific activity curve to look like? You may assume that:

$$Y_{X/S} = 0.5 \qquad Y_{X/N} = 10$$

$$Y_{X/O} = 1.0$$

Area of bacteria $\approx 5 \times 10^{-8} cm^2/cell$

Weight of bacteria $\approx 10^{12}$ g/cell

Desired final cell conc. = 10 g/L

$\mu_{max} \approx 0.2 h^{-1}$

cells are 60% protein

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