

# **Bioreactor Design Fundamentals**

---

**Norton G. McDuffie**

University of Texas at Austin

**Butterworth-Heinemann**

Boston London Oxford Singapore Sydney Toronto Wellington

Copyright © 1991 by Butterworth-Heinemann, a division of Reed Publishing (USA) Inc. All rights reserved.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior written permission of the publisher.

Recognizing the importance of preserving what has been written, it is the policy of Butterworth-Heinemann to have the books it publishes printed on acid-free paper, and we exert our best efforts to that end.

**Library of Congress Cataloging-in-Publication Data**

McDuffie, Norton G.

Bioreactor design fundamentals / Norton G. McDuffie.  
p. cm.

Includes bibliographical references and index.

ISBN 0-7506-9107-7  
1. Bioreactors—Design and construction. I. Title.  
TP248.25.B55M33 1991

660'.6—dc20

91-15602  
CIP

**British Library Cataloguing in Publication Data**

McDuffie, Norton G.

Bioreactor design fundamentals.  
I. Title  
660.63

ISBN 0-7506-9107-7

Butterworth-Heinemann  
80 Montvale Avenue  
Stoneham, MA 02180

10 9 8 7 6 5 4 3 2 1

Printed in the United States of America

To my wife, Carole, who has endured with love and patience throughout the preparation of this manuscript.

# Preface

---

The disciplines of biochemical engineering and industrial microbiology have advanced tremendously during the past few years. Most important applications in these areas involve utilization of one or more biological reactor systems. Consequently, a large volume of literature has developed, with biological reactor design as an integral part of countless journal articles and comprehensive books. In teaching courses in bioengineering, I have noted the need for a unified coverage and condensation of applications of biological kinetics and thermodynamics, combining concepts and methods common to the many specific systems. Such a condensation has already taken place in chemical reactor design as a specialty of chemical engineering practice. This monograph certainly is a precursor to others because of the rapid rate of development in the state of knowledge in the bioreactor field. However, many of the basic concepts are unlikely to change, simply because they work. Usually, more complex models are the outgrowth of new knowledge. Such models will probably be additive to those currently in use rather than complete replacements.

This monograph should be of use to both practicing chemical engineers and industrial microbiologists. It is not meant to replace more comprehensive textbooks and compendia, but it should be of use in upper-level and postgraduate courses in bioreactor design, where a specialized coverage of bioreactor design fundamentals is desired.

*N.G. McDuffie*  
Corvallis, Oregon  
July 1991

# Chapter 1

---

## Introduction

The field of biological reactor design is an extremely active area of research and development. Thus any publication on specific design is in danger of early redundancy. Because of the rapid rate of development in design, many of the detailed descriptions of biological reactors are best left to current journal articles. Hence this monograph is a guide to the central aspects of biological reactor design rather than a comprehensive coverage of the literature. In fact, much of the literature still addresses research and development rather than applied design.

Just as chemical reactor design is central to the practice of chemical engineering, biological reactor design is essential for applications of biochemical engineering and industrial microbiology and important for other areas of biotechnology. As always, carving out a portion of a process and giving it a specific title is difficult. In this monograph, therefore, some arbitrary decisions were necessary to limit the length of the publication and to ensure that the most significant items are covered in sufficient detail. For example, control and optimization are not discussed in detail. They certainly qualify as important aspects of reactor design, but they are probably better covered separately as a category of bioreactor design.

Biological systems are diverse. The current state of the art of genetic manipulation allows further extension of this diversity into previously unimagined areas. Thus the designer should optimize both the reaction system and the physical reactor. Usually, the designer determines the system and conditions and then designs the reactor around them. Ultimately, designs result from coordinated studies of biological systems and conditions, as well as mechanical constructions. The first step in design always is to determine which biological system to utilize. For example, more than one strain of microorganism might be used to carry out a chemical conversion. The best strain for the desired reaction might be improved further by genetic manipulations, such as sexual recombinations or mutations followed by strain selection. Further improvements in selectivity or yield may result from various recombinant DNA techniques involving DNA revision, insertion into more productive organisms, or gene amplification. Project economics, however, may limit the designer to the use of less sophisticated systems. Genetic engineering is still expensive and

## 2 Bioreactor Design Fundamentals

time-consuming. Moreover, the products of such efforts may not be very long-lived relative to the wild-type organisms or natural enzymes.

Many common ties exist between traditional chemical reactors and biological reactors. In their simplest form the basic rate equations for enzyme-catalyzed reactions or for cell reproduction are the same as those for heterogeneous catalysis of fluid reactions. The basic principles of thermodynamics, kinetics, and mass transfer apply to biological reactions. Biological reactions, however, are carried out under much less severe conditions than those used in most nonbiological systems. These differences offer both advantages and disadvantages. Obviously, a great advantage lies in the low heat requirements for biological reactions. Low-temperature operation presents fewer problems with thermal degradation and thus allows greater specificity. Fragility of cells and enzymes limits mechanical processes of mixing and separation. Cell death and enzyme inactivation limit their use to at least some extent in most applications.

Enzyme kinetics are central to all applications of biological reactions, whether they involve cells or enzyme-catalyzed specific reactions. Perhaps the most important distinction of enzymatic reactions is that they have lower activation energies than comparable nonenzymatic reactions (catalyzed or not). In addition, they generally possess greater specificity. Another generally unique factor is that enzyme systems allow coupling of reactions for efficient utilization of free energy. None of the laws of thermodynamics is violated in biological systems, so far as can be established. Living cells utilize energy in open systems to produce low-entropy organizations associated with macromolecular structures of proteins and nucleic acids, as well as the highly structured cells themselves. Steady-state thermodynamics become very important in analysis of these systems. Active cells are never in thermodynamic equilibrium. Likewise, enzymatic reactions are in dynamic, or steady, states. Even enzyme deactivation is best approached by use of nonequilibrium thermodynamics.

Any reactor that contains materials undergoing biological change can be considered a biological reactor. The terms, *biological reactor* or *bioreactor*, are preferable to the more specific—although not yet consistently applied—terms *biochemical reactor*, *fermenter* (or *fermentor*), and so on. Design of biological reactors involves determining operating conditions, sizing the reactor, designing for mixing and mass transfer, controlling temperature and sterility, determining the means of feed introduction and product removal, and controlling operating variables, such as pH, oxygen concentrations, and illumination. At present, the designer must determine many of the requirements for production units by progressively scaling up from bench-scale units. This approach applies particularly to determining mixing and mass-transfer parameters. In many cases kinetic data are available only for initial rates of reaction for pseudo first-order enzymatic reactions or for batch cultures in the case of cell growth kinetics.

The first requirement for bioreactor sizing is to develop good kinetic models over the entire range of expected reactor conditions. In the past, designers sized bioreactors for cell culture by using batch culture data, even though actual conditions were far removed from those present in the batch systems. Designers

used simplified kinetic expressions for the kinetics of enzyme-catalyzed reactions, because analytic solutions of more complex models were difficult or impossible to obtain. Of course, more complex models are not generally justified when simpler models describe the systems adequately. However, the availability of inexpensive computer systems for solving complex partial differential equations now means that the complexity of mathematical models should no longer limit their application in the design of biological reactors. The ultimate goal of developing general kinetic and transport models that can be used for untested biological systems remains unachieved. Unfortunately empirical models still must be used, even for systems that have been studied extensively. Rapid progress is being made in related research, however, and the designer should make every effort to use the best models available within economic constraints. Although covering all the exciting developments in the field is impossible here, changes that are expected to affect various specialized areas of bioreactor design and replace current practice are indicated.

# Chapter 2

---

## Thermodynamics and Stoichiometry for Bioreactor Design

### THERMODYNAMICS

Biological processes are irreversible, as are nonbiological processes. Even though complex reactions taking place are irreversible, or steady state, equilibrium thermodynamics help explain them. In fact, equilibrium thermodynamics are used in many aspects of modeling and design. From a thermodynamic standpoint, biological reactions are unique, because many enzymatic processes can be accomplished by direct coupling of reactions having negative Gibbs free-energy changes to those having positive Gibbs free-energy changes. Further, many enzyme systems can catalyze sequential reactions, as well as highly stereospecific reactions. Also, cells can reproduce their own synthetic systems for essentially perpetual maintenance of steady-state existences. These highly organized, low-entropy states are maintained by input of energy from the environment and by a net increase in entropy overall. For photosynthetic systems, high-level energy is obtained from sunlight (or a suitable substitute). For others it is obtained from chemical bond energy in inorganic or organic molecules.

### Standard States

Biochemical thermodynamics differ slightly from classical thermodynamics in that the standard state for reactions in aqueous solution is at pH 7.0 rather than at pH 0.0. In addition, for most practical purposes, the standard-state concentration for reactants and products in solution may generally be set at 1.0 M, and molar concentrations may be used for most free-energy and equilibrium calculations for the dilute solutions involved. These assumptions are made here unless otherwise noted. For thermodynamic properties and changes of thermodynamic properties at standard biological states, the general conven-

## 6 Bioreactor Design Fundamentals

tion is to add a prime notation to the appropriate symbol to indicate that the standard biological state is being used in place of the standard chemical state.

### pH and Buffers

The importance of pH considerations in biological reactor and process design cannot be overemphasized. Most biological reactions are sensitive to pH values as they affect the molecular structures involved in enzymes and/or reactants and as they affect energetics of the reactions. For most ionization reactions, equilibrium is rapidly attained compared to other reactions. Thus equilibrium assumptions can be used for the ionizations. Furthermore, most biological reactions are carried out in relatively dilute solutions. Thus the concentration of water may be assumed constant at about 55.5 M and so be incorporated in reaction equilibrium constants when water is one of the substances used. The equilibrium relationship for a weak acid is

$$K_a = \frac{[H^+] [A^-]}{[HA]}, \quad (2.1)$$

or in its logarithmic form (the Henderson–Hasselbalch equation),

$$pH = pK_a + \log_{10} \frac{[A^-]}{[HA]}, \quad (2.2)$$

where  $K_a$  is the acid dissociation constant and  $pK_a$  is  $-\log_{10} K_a$  for the ionization reaction,



Almost all biochemical systems involve some ionized species that may be treated as weak Bronsted acids or their conjugate bases, even though multiple ionizations may be involved. Therefore the ionization equilibrium calculations are of some importance, not only in buffer and pH calculations, but also in determinations of biomolecular states. The shape of the pH curve for titration of mixtures of weak acids and their conjugate bases shows that pH changes more gradually at values near the  $pK_a$  value. This fortunate circumstance allows some stability in control of pH of the aqueous environment through the use of buffers having appropriate  $pK_a$  values. Appropriate buffering should always be one of the prime considerations in design of a biological reaction system. A dilute buffer solution—or one with a  $pK_a$  value far removed from the desired pH operating value—is not effective, because it allows wide swings in pH values when a strong acid or base is added in an attempt to control the pH. Temperature has an effect on the weak-acid ionization equilibrium and thus on  $K_a$  and pH values (Lewis and Randall, 1961). Appropriate  $K_a$  or  $pK_a$  values

for system conditions must therefore be used. Corrections for temperature and ionic strength effects on acid and water dissociation constants are necessary in all cases for more exact solutions. They are even required for acceptable solutions when ionic strength and/or temperature extremes are encountered. Such corrections for the CO<sub>2</sub>-water system are discussed later in this chapter in the section on Phase Equilibrium Relationships.

## Stoichiometry

Any treatment of thermodynamics of conversion processes requires material-balance calculations. Basic to all balances is an elemental balance, because the elements themselves are not converted. In simple conversions following defined equations, conventional molar balances based on reaction stoichiometry may be utilized. For polymers, stoichiometric balances must refer to populations of polymeric molecules of differing molecular weights. For systems involving cells, matters become more complex because of the variety of compounds present and the variable nature of the cells themselves. A convenient unit of material that can be used in biological reactions from the simplest to the most complex is the C-mole of organic compound or mixture of carbon compounds. The *C-mole* is the amount of material containing one gram atom of carbon. Thus a C-mole of glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) is the unit CH<sub>2</sub>O. Traditionally, the empirical formula for living cells is defined in terms of the major elements contained in the dried cells. A typical C-mole empirical formula, which has been used extensively for a dry mass of microorganisms, is CH<sub>1.8</sub>O<sub>0.5</sub>N<sub>0.2</sub> (Roels, 1983a). If further information concerning the ash content of dry cell weight is known, approximate material and energy balances can be determined. An average ash content of 9% of dry weight may be used for many bacterial cells. Thus for one C-mole with the simplified formula CH<sub>1.8</sub>O<sub>0.5</sub>N<sub>0.2</sub> and a formula mass of 24.6, the ash content would be (0.09/0.91) (24.6 g) = 2.4 g, and the C-mole total dry mass would be approximately the sum of the ash and C, H, O, and N masses, or 27.0 g. Such calculations are only approximations, because they describe a continually varying population and because the calculations do not account for volatile elements, especially sulfur; however, they are useful for initial design estimates.

The overall material balance must account for conversion of compounds to cellular material and complex secondary products and for energy-yielding reactions. The majority of applications involve conversion of a substrate in one or more energy-yielding reactions to provide for conversion of some of the same substrate (usually) to cellular material and secondary products. The amount of substrate that must be used to provide energy depends on the nature of the energy-yielding reaction(s), the energetic and stoichiometric requirements for incorporation of substrate into complex cells and products, and the maintenance requirements for a continuing steady state of existence. The relations are covered in detail by Roels (1983a,b), who developed correlations

for estimating some of the important thermodynamic parameters for cells and metabolic reactions. The correlations that can be used to predict yields, substrate and oxygen requirements, and heat dissipation are of value in designing and scaling up and in analyzing laboratory and production data.

## Energy Balances

As aerobic energy-yielding reactions are ultimately oxidation reactions—usually with molecular oxygen as the electron acceptor—energies of combustion perhaps are more convenient to use than energies of formation. Organic compounds contained in substrates, cells, and products may be rated according to their *degree of reduction*, with NH<sub>3</sub> as a nitrogen source and elemental constituents other than carbon, hydrogen, and nitrogen assumed to have negligible effects. According to Roels (1983 a,b), the degree of reduction,  $\gamma_i$ , for this case is

$$\gamma_i = 4 + a_i - 2b_i - 3c_i, \quad (2.4)$$

where  $a_i$  = number of gram atoms of hydrogen per C-mole of component  $I$ ;

$b_i$  = number of gram atoms of oxygen per C-mole of component  $I$ ;

$c_i$  = number of gram atoms of nitrogen per C-mole of component  $I$ .

The approximate values for the standard-state heat of combustion,  $\Delta h_{ci}^\circ$ , and the standard-state Gibbs free energy of combustion,  $\Delta g_{ci}^\circ$ , for a C-mole of component  $I$  are as follows:

$$\Delta h_{ci}^\circ = 115 \gamma_i \pm 18 \text{ kJ/C-mole}, \quad (2.5)$$

and

$$\Delta g_{ci}^\circ = (94.4\gamma_i + 86.6) \pm 18 \text{ kJ/C-mole}. \quad (2.6)$$

For most naturally occurring reactions, the change in Gibbs free energy of the system upon combustion is negative. Roels follows the convention of assigning a positive value for heat of combustion and, consequently, for Gibbs free energy of combustion for energy-yielding combustion reactions. Roels (1983a,b) further showed that the maximum thermodynamic efficiency for cell growth is about 65%.

Anaerobic processes depend on conversion of large amounts of substrate to product, and cellular capture of available free energy is largely the result of entropy increases from substrate conversion to simple products. Use of thermodynamic predictive equations for anything more than approximate values of heat effects or thermodynamic efficiency of cell production is not currently worthwhile. Predicting cell yield from knowledge of relative ATP production

of the specific catabolic processes offers more chance for success. For aerobic production of cells with complete conversion of substrate to cells, carbon dioxide ( $\text{HCO}_3^-$ ), and water, Roels (1983a,b) defines thermodynamic efficiency of a steady-state growth system as

$$\eta_{th} = \frac{\Phi_x \Delta g_{cx}^\circ}{\Phi_s \Delta g_{cs}^\circ + \Phi_N \Delta g_{cN}^\circ} \quad (2.7)$$

where  $x$  designates cells;  $s$ , substrate; and  $N$ , nitrogen; and

where  $\eta_{th}$  = thermodynamic efficiency;

$\Phi_N$  = net rate of  $\text{NH}_3$  flow into system, mole/s;

$\Phi_x$  = net rate of cell flow out of system, C-mole/s;

$\Phi_s$  = net rate of substrate flow into system, C-mole/s.

The general correlations then (for noncarbon-limited growth) are

$$\frac{D}{\Phi_x} = \Delta g_{av/e}^{\circ'} \left( \frac{25.8}{Y_{av/e}} - 4.8 \right) + 65.8, \quad (2.8)$$

where  $D$  = heat dissipation, kJ/s;

$\Delta g_{av/e}^{\circ'}$  = average Gibbs free-energy change per mole of electrons transferred in substrate conversion to products, kJ/mol;

$Y_{av/e}$  = cell yield in grams of dry mass cells per mole electrons transferred in substrate reaction to products.

In addition,

$$Y_{av/e} = 5.85 \eta_{th}, \quad (2.9)$$

and

$$\Phi_H = 460 \Phi_O, \quad (2.10)$$

where  $\Phi_H$  = heat production rate, kJ/s;

$\Phi_O$  = net  $\text{O}_2$  uptake rate, mol/s.

If cell growth is carbon limited, efficiency predictions become more difficult, because in carbon-limited growth the amount of substrate required by cells is determined by the requirement for cellular carbon. This requirement holds even though energy in excess of that required for growth and maintenance is available in each C-mole of substrate required for carbon.

As mentioned previously, the heat dissipation for anaerobic growth is much smaller than that for aerobic growth. For example, Roels (1983b) determined

that aerobic growth of yeast generates about 300–350 kJ/(C-mole yeast), whereas the anaerobic growth process generates only about 90 kJ/(C-mole yeast). Thus removal of metabolic heat is not a major problem for anaerobic processes. On the contrary, heat may have to be added to maintain desired operating temperatures in cold climates or when there is no major input of mechanical mixing energy. Yield factors for anaerobic growth are directly related to ATP generation by the catabolic processes utilized by the cells for conversion of substrate to products. However, as Stouthamer and Van Verseveld (1985) observed, generalizations are difficult to make. Yield relationships are important, and they must be based on solid laboratory determinations, not generalizations. Factors for aerobic or anaerobic processes as determined for growth phases differ from those attained in cell phases, where energy requirements for cell maintenance become appreciable or where alternative metabolic pathways are utilized. Yield factors also vary as conditions of the following change: substrate, product, and inhibitor concentrations, pH, cell age distribution, redox potential, and oxygen tension. Thus production yields may not match laboratory yields. The designer must take care to duplicate the expected production conditions as closely as possible when making laboratory runs for use in scaling-up. Exact duplication is not practical in most cases.

The high ratio of water to reactants aids in the control of temperature in most biological reactors. Heat removal becomes a major problem, however, in vigorous aerobic conversions of highly reduced substrates. The overall rate of heat production,  $Q$ , within the system is as follows for a steady-state flow reactor:

$$Q = \sum_{i=1}^n \Phi_i h_i + P + D, \quad (2.11)$$

---

where  $Q$  = rate of heat production, kW;

$\Phi_i$  = vector rate for stream  $I$ , positive for input and negative for output, mol/s;

$h_i$  = enthalpy of stream  $I$ , kJ/mol;

$P$  = mechanical power input, kW;

$D$  = dissipation rate for metabolic processes, kW;

$n$  = number of individual streams.

The term  $Q$  can be positive or negative, depending on whether the reactor is cooled or heated, respectively. The heat balance is straightforward, but some important points are often overlooked. The term  $\sum_{i=1}^n \Phi_i h_i$  includes pump and gas compressor work used in gas-driven mixing and in static mixers using feed energy (rather than internal recycle), so long as  $h_i$  is evaluated at reactor inlet conditions for the given stream. The value of  $h_i$  is a quantity corrected to account for the physical heat effects with the value of  $D$  accounting for chemical reaction heat production. The datum for  $h_i$  is best set at reactor outlet tem-

perature and pressure so that produced streams have  $h_i$  values of zero. The inlet stream enthalpies then are given by

$$h_i = \int_{T_o}^{P_i} c_{p_i} dT + \int_{P_o}^{P_i} v_i dP, \quad (2.12)$$

where  $T_o$  = reactor outlet temperature, K;

$T_i$  = temperature of stream  $I$ , K;

$c_{p_i}$  = heat capacity of stream  $I$  at pressure,  $P_o$ , kJ/(mol·K);

$P_o$  = reactor outlet pressure, kPa;

$P_i$  = pressure of stream  $I$ , kPa;

$v_i$  = molar volume of stream  $I$  at temperature  $T_i$ , m<sup>3</sup>/mol.

Equivalent mass units may be used in analogous consistent equations.

### Phase Equilibrium Relationships

The primary phase equilibria subject to control in bioreactor designs are those involving vapor–liquid systems—most specifically, vapor phases in contact with aqueous systems. Oxygen and carbon dioxide are transferred between phases in many processes. Oxygen concentration in the aqueous phase can be important in oxygen-limited aerobic systems. Carbon dioxide acts as a weak acid in aqueous solution; thus it has a direct effect on the pH of many systems. Volatile products, such as ethanol, can be lost in gases from fermentation processes. As closer control of bioreactor systems becomes more and more important, precise mathematical relationships describing vapor–liquid equilibria become essential for control.

Volatile liquid-phase constituents can be lost to reactor gas phases with improper control of conditions or excessive gas flow rates. Generally, dissolved volatile liquids cannot be treated as constituents of ideal solutions. Certain liquids (e.g., alcohols and esters) usually form nonideal solutions in water—especially in solutions with salts and other dissolved species. Raoult's law is inadequate for use in vapor–liquid equilibrium calculations for such systems. Liquid-phase activity coefficients can be used to estimate vapor–liquid equilibrium concentrations for binary mixtures, such as ethanol and water. For low-pressure systems, partial pressures of components can be determined by use of the relationship:

$$p_i = (VP_i)\gamma_i x_i, \quad (2.13)$$

where  $p_i$  = partial pressure of component  $I$  in vapor phase, kPa;

$VP_i$  = vapor pressure of pure component  $I$  at system temperature, kPa;

$\gamma_i$  = liquid–phase activity coefficient of component  $I$ ;

$x_i$  = mole fraction of component  $I$  in liquid phase.

For example, the value of  $\gamma_i$  for ethanol in water at 25° C is 2.85 for an ethanol concentration of 10 mol %, as predicted using the Van Laar equation with constants from Chao and Greenkorn (1975). Thus the use of Raoult's law would underestimate the mole fraction of ethanol in the vapor phase by a factor of 1/2.85. In addition to Chao and Greenkorn's work, Reid, Prausnitz, and Poling (1987)—or any other good thermodynamics reference—presents further details and methods of calculation for high-pressure systems. Convenient commercial computer programs also are available for determining vapor–liquid equilibria for nonideal systems by appropriate thermodynamic models. Even though most do not contain corrections for ionic strength or specific ion constituents of the aqueous phase, they certainly provide a closer approach to the actual system with a tremendous time saving over hand calculations. Two specific vapor–liquid equilibrium systems deserve special attention because of their importance in so many biological systems: the oxygen–air–water system and the carbon dioxide–water system.

The oxygen–water equilibrium is of concern both in aerobic and anaerobic systems. Because oxygen is only slightly soluble in water, biological oxidation reactions and aerobic cell growth can be limited by restricting the availability of dissolved oxygen. Anaerobic systems, however, may be inhibited by dissolved oxygen, so oxygen depletion should be emphasized. Generally, the rate of oxygen transport from the gas phase to the liquid phase is controlled by liquid-phase mass transfer resistance. Treatment of the mass transfer problem then is usually carried out by ascribing mass transfer rates to rates through a liquid film at the gas–liquid interface with the interfacial liquid concentration of oxygen taken to be the concentration in equilibrium with the gas-phase concentration. Thus accurate prediction of oxygen–water equilibrium becomes important in mass transfer rate computations, as well as in determinations of dissolved oxygen limitations. Water vapor pressure affects transfer of water to or from the gas phase. A design is not complete unless it provides an evaluation of water transfer by evaporation or absorption and corrects any problems caused by uncontrolled water transport. Water evaporation can cause undue increase in salt concentration of media and drying of exposed surfaces, especially in highly aerated systems. Presaturation of the air with water for prevention of such effects is advisable.

Temperature and salinity (or ionic strength) must be corrected in order to predict oxygen solubility in aqueous systems with any degree of accuracy. Relationships developed from Chen and Carpenter's (in Battino, Rettich, and Tominaga, 1983) modification of the Weiss (1970) equation as revised further are

$$C = \exp \left[ -1278.9956 + \frac{36063.19}{T} + 220.1832 \ln T - 0.351299T + \left( 6.229 \times 10^{-3} - \frac{3.5912}{T} \right) S + 3.44 \times 10^{-6} S^2 \right]; \quad (2.14)$$

$$C_1 = 22414C; \quad (2.15)$$

$$C_2 = 3200C; \quad (2.16)$$

where  $C = O_2$  concentration in water, M;

$C_1 = O_2$  concentration in water, mL(STP)/L;

$C_2 = O_2$  concentration in water, mass ppm;

$T =$  temperature, K;

$S =$  salinity (in seawater), mass ‰ (parts per thousand).

Equations 2.14–2.16 express solubility in water of oxygen from moist air in equilibrium at a total pressure of 101.325 kPa. The three equations are presented because of the diversity in preferences and in applications of the solubility units. Figure 2.1 shows values of  $C$  obtained from Equation 2.14. The strong effects of both temperature and salinity are evident.

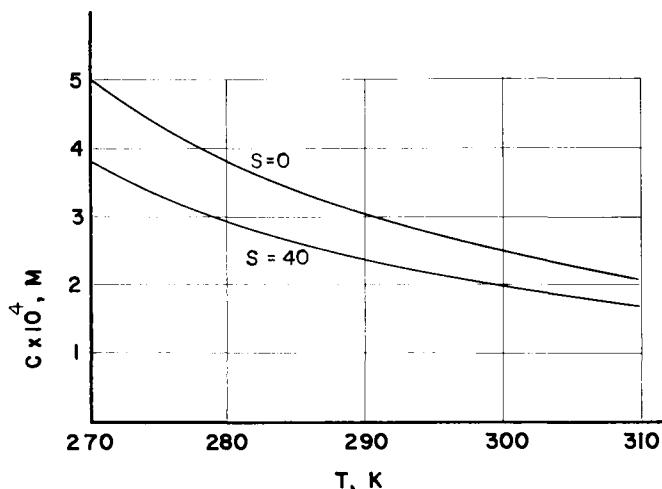
The use of seawater salinity is probably a good general approach to determining the average salt mix in biosystems. Henry's law (for low pressures) is

$$p_i = k_H x_i, \quad (2.17)$$

where  $p_i =$  partial pressure of component  $I$  (water in this case) in the gas phase;

$k_H =$  Henry's law constant, with units of pressure;

$x_i =$  mole fraction of component  $I$  in the liquid phase.



**FIGURE 2.1** Solubility,  $C$ , mol/L,  $O_2$  in water as a function of temperature,  $T$ , and salinity,  $S$ , ‰.

## 14 Bioreactor Design Fundamentals

Equation 2.17 applies to the solubilities shown in Figure 2.1 if  $k_H$  is a function of salinity and, as discussed by Weiss (1970), dry air contains 20.946 mol % oxygen. However, moist air oxygen partial pressure then is

$$p_{O_2} = 0.20946(P_t - VP_{H_2O}), \quad (2.18)$$

where  $P_t$  = total system pressure;

$VP_{H_2O}$  = vapor pressure of water at system temperature.

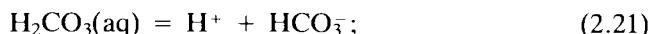
Thus a different value of Henry's constant has to be used for each salinity. Benson and Krause (1976) presented an equation for Henry's constant for  $O_2$  in pure water as a function of temperature:

$$k_H = \exp \left[ 4.0605 + \frac{5.4167 \times 10^3}{T} - \frac{10.261 \times 10^5}{T^2} \right] \text{ atm}, \quad (2.19)$$

where  $T$  is in K.

They also reported the value of the heat of solution of  $O_2$  in water to be 2921 cal/mol. Equation 2.19 gives a close match to the data of Battino, Rettich, and Tominaga (1983) for  $O_2$  in water of zero salinity when  $p_{O_2}$  for moist air is determined by use of Equation 2.18.

Carbon dioxide is a product of many biological reactions and a carbon source and/or reactant in many others. Further, its functions in relation to atmospheric heat radiation and to its effects on acidity of the aquasphere have universal importance. In aqueous solution,  $CO_2$  acts as a weak acid with two ionizations. Calculations of equilibrium concentrations can become quite complex when all factors are taken into account. Butler (1982) presented a clear, in-depth discussion of the calculations needed to predict accurately  $CO_2$  equilibria in many different situations. The equilibria to be considered involve the following reactions:



The equilibrium relationships in the forms used by Butler (1982) are as follows (with concentrations of the species in brackets in mol/L):

$$[CO_2] = K_H P_{CO_2}; \quad (2.23)$$

$$[H^+][HCO_3^-] = K_{a1}[CO_2]; \quad (2.24)$$

$$[H^+][CO_3^{2-}] = K_{a2}[HCO_3^-]; \quad (2.25)$$

where  $[CO_2]$  = total concentration of  $CO_2$  and  $H_2CO_3$  in solution, M;  
 $K_H$  = a constant derived from Henry's law for dilute solutions,  
M/atm;  
 $K_{a1}$  and  $K_{a2}$  = acid dissociation constants for the respective ionizations, M.

The values of equilibrium constants for these equations are strongly affected by temperature and by ionic strength of the medium. Table 2.1 contains selected values from Butler (1991); extended tables are presented in Butler (1991).

The  $pK$  values ( $-\log_{10} K$ ) are convenient to use both for elimination of exponential expressions and for combinations with pH and pOH values. Equations 2.23–2.25 yield concentrations of the various dissolved species in terms of  $pK$  values:

$$\log [CO_2] = -pK_H + \log P_{CO_2}; \quad (2.26)$$

$$\log [HCO_3^-] = -pK_H - pK_{a1} + \log P_{CO_2} + pH; \quad (2.27)$$

$$\log [CO_3^{2-}] = -pK_H - pK_{a1} - pK_{a2} + \log P_{CO_2} + 2pH; \quad (2.28)$$

$$\log [OH^-] = -pK_w + pH; \quad (2.29)$$

where  $pK_w = \log K_w$ ;  
 $K_w = [H^+] [OH^-]$ .

The concentrations of the various ionic species are important in calculations for mass balances and for determining pH of solutions. Munjal and Stewart (1971) published a correlation for determining  $CO_2$  solubility in water and saltwater made with synthetic sea salt:

**Table 2.1** Effect of Temperature and Dissolved Salt on  $CO_2$  Equilibrium Constants

	<i>Constant</i>			
	$pK_H$	$pK_{a1}$	$pK_{a2}$	$pK_w$
<i>Pure Water</i>				
25 °C	1.47	6.352	10.329	13.999
30 °C	1.53	6.327	10.290	13.833
35 °C	1.59	6.309	10.250	13.676
40 °C	1.64	6.298	10.220	13.533
<i>Seawater with Salinity, S = 35‰</i>				
25 °C	1.54	5.86	8.95	13.20

Source: Selected values adapted from J. N. Butler, *Carbon Dioxide Equilibria and Their Applications*, Lewis Publishers 1991, p. 16. (Reproduced with permission of the author and publisher.)

$$\ln(x) = \ln(P) - \left\{ \left[ (16.43 + 0.40S_1^{0.595}) - \frac{2698 + 36S_1}{T} \right] - \left( \frac{P}{R} \right) \left[ \frac{203.14}{T} - \frac{9.756 \times 10^4}{T^2} - (e^{-(0.390 + 2S_1)} + 1) (3.95 \times 10^{-3}R) \right] + \left( \frac{P}{R} \right)^2 \left( \frac{119.76}{T} + \frac{552.22}{T^2} - \frac{3.964 \times 10^7}{T^3} + \frac{9.517 \times 10^8}{T^4} \right) \right\}, \quad (2.30)$$

where  $P$  = system pressure, atm;

$T$  = temperature, K;

$S_1$  = dimensionless salinity,  $S/34.4$  mass %;

$R$  = universal gas constant,  $82.0053 \text{ (cm}^3\text{-atm)/(mol}\cdot\text{K)}$ ;

$x$  = mole fraction  $\text{CO}_2$  dissolved in liquid phase.

Equation 2.30 was developed to correlate data for the  $\text{CO}_2\text{-H}_2\text{O}$  system, but it should be applicable to calculations of Henry's law constants for use in systems containing other gases, especially at low pressures.

## Reaction Equilibrium

Perhaps the most important calculations concerning reaction equilibrium limitations in bioreactor design are those related to denaturation of proteins, especially those composing the apoenzyme structures. The unique activities and structures of the proteins (and nucleic acids) are directly related to their complex intramolecular interactions, such as formation of hydrogen bonds and salt bridges. Because of the large number of such interactions, a great amount of energy must be supplied to derange the unique structures, which represent low entropy states. The mathematical treatment of equilibrium in such systems usually involves minimization of Gibbs free energy for the system. For single reactions, the equivalent use of equilibrium constants replaces this exercise. For the representative reaction,



the equilibrium constant for dilute solutions can be approximated as

$$K_e = \frac{[\text{C}]^c[\text{D}]^d}{[\text{A}]^a[\text{B}]^b}. \quad (2.32)$$

The equilibrium constant,  $K_e$ , is related to the thermodynamic parameters of the reaction as follows:

$$K_e = \exp\left[\frac{-\Delta G^{\circ'}}{RT}\right], \quad (2.33)$$

where  $\Delta G^{\circ'}$  = Gibbs free-energy change for stoichiometric reaction at biological standard state and system temperature, J/mol;

$R$  = universal gas constant, 8.314 J/(mol·K);

$T$  = temperature, K.

The following relationships for free-energy change help in the evaluation of the temperature and pressure dependence of  $K_e$ :

$$\Delta G^{\circ'} = \Delta H^{\circ'} - T \Delta S^{\circ'}, \quad (2.34)$$

$$\frac{\partial(\ln K_e)}{\partial T} = \frac{\Delta H^{\circ'}}{RT^2}, \quad (2.35)$$

or

$$\frac{\partial(\ln K_e)}{\partial(1/T)} = \frac{-\Delta H^{\circ'}}{R} \quad (2.36)$$

Equations 2.34–2.36 are used in discussions of temperature effects on reversible denaturation of enzymes and the resultant effects on enzyme activity and rate of the catalyzed reaction. The same treatment is used for temperature effects on biological growth rates. For high pressures, consideration must be given to the effect on equilibrium, not on changes in  $K_e$ .

Chemical equilibrium studies are important in relation to limits on biochemical reactions, redox potential relationships, and other specific parameters of bioreactors. In general, however, these extensions currently are the subjects of specialized research and development.

# Chapter 3

## Enzyme Kinetics

Enzyme kinetics are the bases of most reactor designs. Even in those situations where mass-transfer rate controls rate of reaction, the intrinsic catalyzed reaction rate must be evaluated to establish the rate expression. The designer must either incorporate both the reaction rate and mass-transfer rate or neglect the reaction rate with justification by comparison with mass-transfer rate.

### MATHEMATICAL MODELS FOR ENZYME-CATALYZED REACTIONS

Symbolism and terminology in enzyme kinetics have been standardized through efforts of the Nomenclature Committee of the International Union of Biochemistry (NC-IUB, 1982) to link them with recommendations of the International Union of Pure and Applied Chemistry (IUPAC, 1981). Rate of reaction for the general reaction can be defined in terms of rate of disappearance of any reactant or rate of appearance of product. The defining equation is

$$\nu = \frac{1}{v_i} \frac{d[I]}{dt}, \quad (3.1)$$

where  $\nu$  = reaction rate in mol/(L·s);

$[I]$  = concentration of component I, either reactant or product (not to be confused with inhibitor), mol/L;

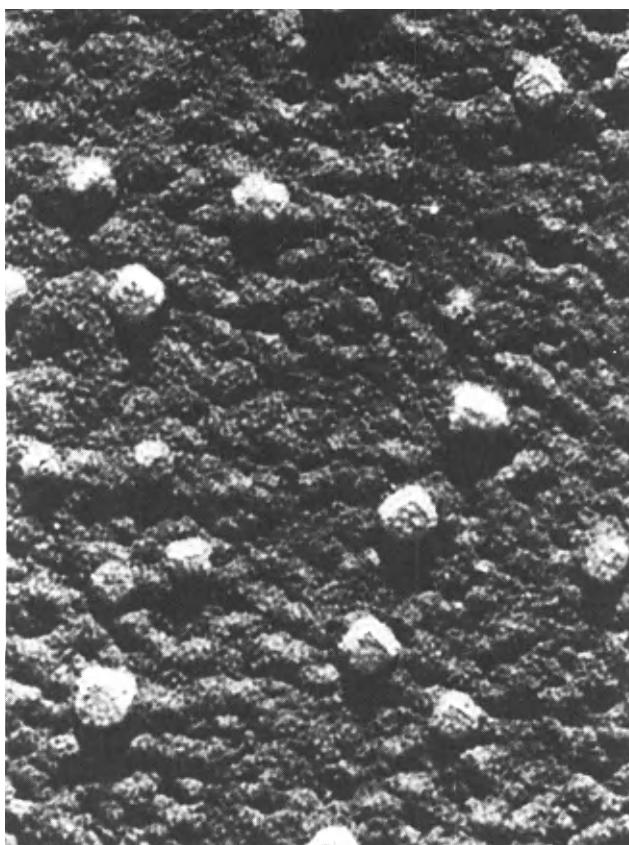
$v_i$  = stoichiometric coefficient of component I, with negative sign for reactants and positive sign for products;

$t$  = time, s.

Many practical enzymatic reactions are reversible or are inhibited by products. Yet rate equations and related constants may be available only for initial rates of reaction. Furthermore, laboratory research results may be interpreted by use of simple models not correctly describing kinetics for the extent of reaction expected in equipment design. Using the simplest equations in reactor design may be satisfactory or even desirable, but the designer should ascertain that they actually work for the conditions and conversions expected in the reactor.

Enzymes are usually large compared to the substrates on which they act. Their activities and specificities depend on their primary structures and on their overall quaternary structures when they act in multienzyme complexes. Their catalytic activities also depend on the presence of proper cofactors, activators, and coenzymes in sufficient concentrations. The extreme organization of some of the more complex systems is exemplified by the pyruvate dehydrogenase complex, which is responsible for conversion of pyruvate to acetyl CoA. This enzyme complex, shown in Figure 3.1, is a highly organized structure made up of a number of subunits (Fernandez-Moran *et al.*, 1965). Obviously, small-molecule kinetic models do not satisfactorily explain the activity of such a large system.

The equations most commonly used as models for overall enzyme kinetics



**FIGURE 3.1** Molecular units of pyruvate dehydrogenase complex from *E. coli*. These 30 nm units contain 5–6 nm subunits seen protruding from the particle at the right of center. Preparation was freeze-dried with liquid nitrogen condenser and shadowed with palladium. Magnification: 125,000 $\times$ . Photograph by Norton McDuffie with the support of Clayton Foundation Biochemical Institute, 1961.

are similar to those used in modeling heterogeneous catalysis, or so-called Langmuir kinetics. The models are based on steady-state formation of enzyme–reactant (enzyme–substrate) complexes and, in the more complicated inhibition models, various enzyme–substrate–inhibitor combinations. Kinetics explained by models developed by such treatments are generally called Michaelis–Menten kinetics. For a single substrate, A, the Michaelis–Menten (or Henri) equation is

$$v = \frac{V[A]}{K_{mA} + [A]}, \quad (3.2)$$

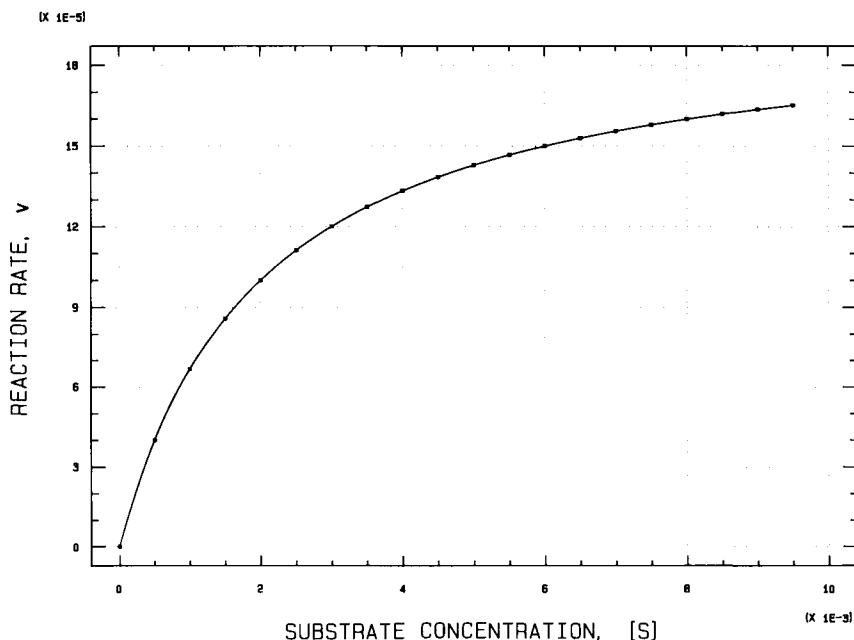
where  $K_{mA}$  = the Michaelis constant for A, mol/L;

$V$  = limiting rate, M/s;

$[A]$  = (or, alternatively,  $[S]$ ) concentration of substrate, A (or S), mol/L.

Figure 3.2 shows a plot of Equation 3.2. In more elementary terms, Equation 3.2 is

$$v = \frac{k_o k_A [E]_0 [A]}{k_o + k_A [A]}, \quad (3.3)$$



**FIGURE 3.2** General form of the Michaelis–Menten plot for irreversible conversion of a single substrate.

where  $[E]_0$  = stoichiometric concentration of active centers, mol/L;

$k_o$  = catalytic constant,  $s^{-1}$ ;

$k_A$  = specificity constant, L/(mol·s).

In Eq. 3.3  $k_o[E]_0$  is equivalent to  $V$  in Equation 3.2. Thus  $k_o/k_A$  is equivalent to the Michaelis constant,  $K_{mA}$ . The two extremes of conditions of substrate concentration in Equation 3.3 are important in many practical cases. When the substrate concentration is low, the reaction is approximately second order, because

$$v \approx k_A[E]_0[A], \quad (3.4)$$

and when the substrate concentration is high, the reaction is essentially zero order with respect to substrate and first order with respect to enzyme, as

$$v \approx k_o[E]_0. \quad (3.5)$$

As specified by the Nomenclature Committee (NC-IUB, 1982), a reaction is classified as one obeying Michaelis–Menten kinetics if the rate equation can be stated in the form

$$v = \frac{[E]_0}{\frac{1}{k_o} + \frac{1}{k_A[A]} + \frac{1}{k_B[B]} + \dots + \frac{1}{k_{AB}[A][B]} + \dots + \frac{[Z]}{k_A^Z[A]} + \dots}, \quad (3.6)$$

where B represents a second substrate, Z represents a product and

$k_B$  = specificity constant for B,  $M^{-1}s^{-1}$ ;

$k_A^Z$  = reciprocal Dalziel coefficient,  $s^{-1}$  in this case;

$k_{AB}$  = reciprocal Dalziel coefficient,  $M^{-2}s^{-1}$  in this case.

Equation 3.6 must have the first two terms of the denominator, but the other denominator terms may or may not be present, depending on the reaction. Equations of this form can be applied to some types of inhibition and to some types of kinetics not usually considered to be of the Michaelis–Menten type; however, some of these special cases are treated here with their own specific nomenclature to minimize confusion. For the special cases when [B] and [Z] are constant (or zero)—or when only the first two terms of the denominator apply—integration of Equation 3.6 is straightforward. The general integral form for these special cases only is

$$\left\{ \frac{1}{k_o} + \frac{1}{k_B[B]} \right\} \left\{ [A] - [A]_0 \right\} + \left\{ \frac{1}{k_A} + \frac{1}{k_{AB}[B]} + \frac{[Z]}{k_A^Z[A]} \right\} \ln \frac{[A]}{[A]_0} = v_A [E]_0 t, \quad (3.7)$$

where  $t$  = lapsed time of reaction, s;

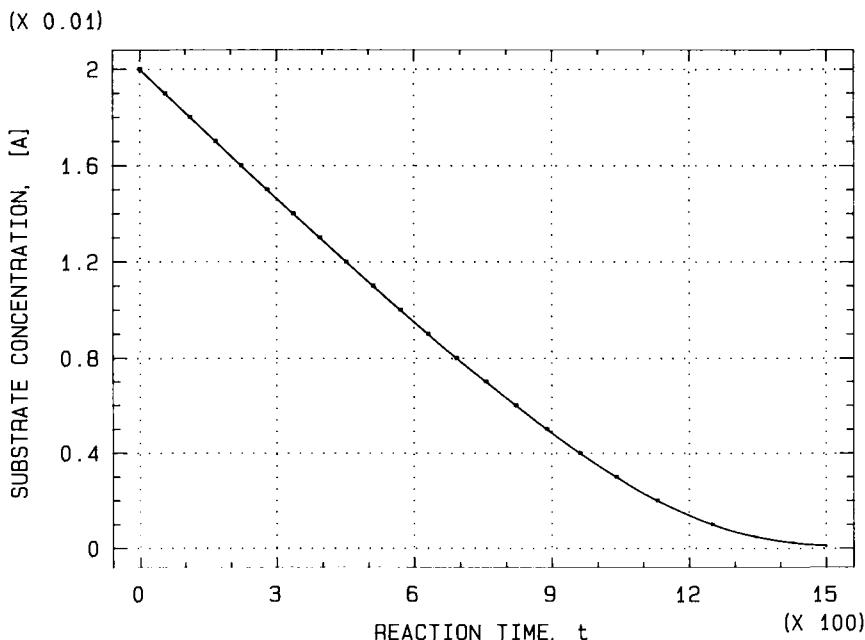
$v_A$  = stoichiometric coefficient for A, with a negative sign, as A is a reactant.

If B and Z have no effect on reaction rate, Equation 3.7 is simplified by eliminating terms containing [B] and [Z]. In the simplified form of Equation 3.2, the integral relationship is

$$K_m v_A \ln \frac{[A]_0}{[A]} + [A]_0 - [A] = Vt. \quad (3.8)$$

This integral form of the Michaelis–Menten equation for a single substrate undergoing an irreversible reaction is illustrated in Figure 3.3. Perhaps the most important attribute of this plot is that most of it is linear. Most industrial reactions occur in this linear region, where the reaction is essentially zero order with respect to substrate. If B undergoes conversion in the same reaction as A, a stoichiometric relationship may be applied in Equation 3.6:

$$[B] = [B]_0 + \left( \frac{v_B}{v_A} \right) \{ [A] - [A]_0 \}. \quad (3.9)$$



**FIGURE 3.3** Integral form of the Michaelis–Menten equation for a single substrate undergoing an irreversible reaction.

An analytical integral also may be developed in such cases. For the simple irreversible reaction of A and B present in stoichiometric proportions or bimolecular reaction of A, in either case without any product effects on reaction rate, the rate expression may be simplified, as shown later. Any further complexity generally leads to the necessity for numerical integration.

Of course, many practical enzyme-catalyzed reactions are reversible, and the kinetics can become quite complex. In general, developing equations for the forward and reverse reactions and expressing the net reaction rate as the sum of the forward rate minus the reverse rate probably are preferable to developing an overall rate equation directly. In many cases, however, equations based on relatively simple mechanisms are sufficient. Haldane (1965) showed that the hydrolysis of  $\beta$ -methylglucoside by  $\beta$ -glucosidase follows a model based on such a mechanism. The form of the general equation developed by Haldane for reversible conversion in a first-order forward and first-order reverse combination is

$$v = \frac{K_1[E]_0([A] - [A]_{eq})}{K_2 + K_3([A]_0 + [P]_0) + K_4([A] - [A]_{eq})}, \quad (3.10)$$

where  $[A]$  = concentration of reactant, M;

$[A]_0$  = initial concentration of reactant, M;

$[A]_{eq}$  = equilibrium concentration of reactant, M;

$[E]_0$  = total enzyme concentration, M;

$[P]_0$  = initial concentration of product, M;

$K_1$  = constant,  $M^{-1}s^{-1}$ ;

$K_2$  = constant;

$K_3$  = constant,  $M^{-1}$ ;

$K_4$  = constant,  $M^{-1}$ .

The constants in Equation 3.10 can be related to the specific reaction rate constants in elementary reactions of the steady-state model of the overall reaction (Haldane, 1965). The reaction rate,  $v$ , is either positive or negative, depending on the state in relation to equilibrium conditions. Equation 3.10 can be integrated most conveniently in terms of the functions

$$z = [A] - [A]_{eq}, \quad (3.11)$$

and

$$a = [A]_0 + [P]_0, \quad (3.12)$$

where  $z$  is the *approach to equilibrium*. The integral relationship then is

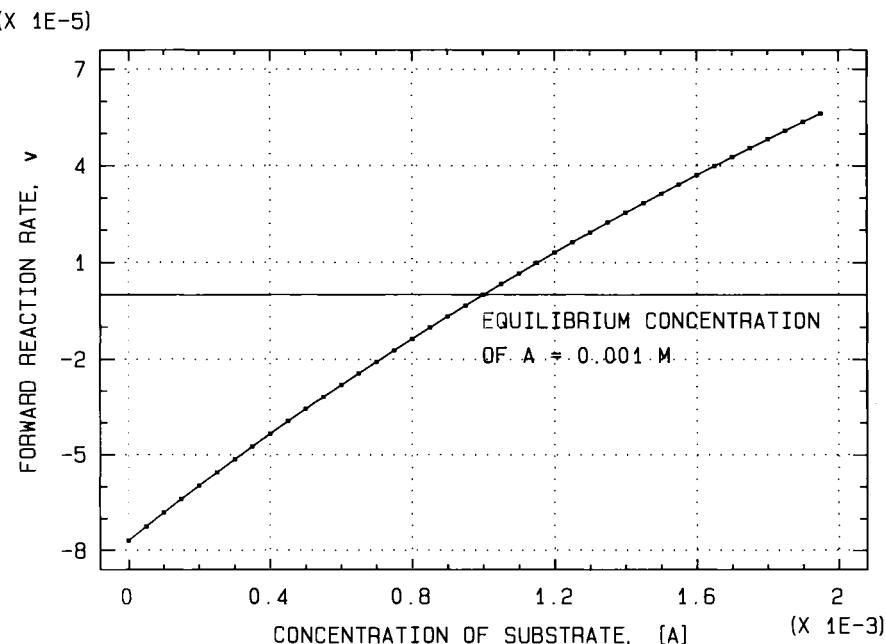
$$(K_2 + K_3a)\ln\left(\frac{z_1}{z_t}\right) = K_1t \quad (\text{for } K_4 = 0), \quad (3.13)$$

and

$$(K_2 + K_3a)\ln\left(\frac{z_1}{z_t}\right) + k_4(z_1 - z_t) = K_1t \quad (\text{for } K_4 \neq 0), \quad (3.14)$$

where  $z_1$  = initial value of  $z$ , M;  
 $z_t$  = value of  $z$  at time  $t$ , M.

Equations 3.13 and 3.14 show that linear or logarithmic functions predominate, so portions of plots of  $[A]$  versus  $t$  are linear or logarithmic. Regardless of whether  $z_1$  is positive or negative,  $z$  will approach zero asymptotically with time. The forms of the specific reaction curves depend on relative values of rate constants and initial concentrations. As Haldane pointed out, the rate versus substrate concentration and the integral substrate concentration versus time curves generally are not symmetrical about the equilibrium point. The rate curve presented in Figure 3.4 shows this asymmetry. With higher concen-



**FIGURE 3.4** Rate curve for one reversible reaction following the form of Equation 3.10 in the vicinity of the equilibrium point.

trations of substrate this particular rate curve levels out as for an irreversible reaction, but when  $[A]$  approaches zero and  $[B]$  increases, the reverse rate never does level out.

In some cases a so-called *rational function* (NC-IUB, 1982) is used in the single-reactant rate equation:

$$v = \frac{\alpha_1[A] + \alpha_2[A]^2 + \alpha_3[A]^3 + \cdots + \alpha_n[A]^n}{1 + \beta_1[A] + \beta_2[A]^2 + \beta_3[A]^3 + \cdots + \beta_m[A]^m}, \quad (3.15)$$

where  $\alpha_i = \text{constant, M}^{(1-i)}\text{s}^{-1}$ ;  
 $\beta_i = \text{constant, M}^{-1}$ .

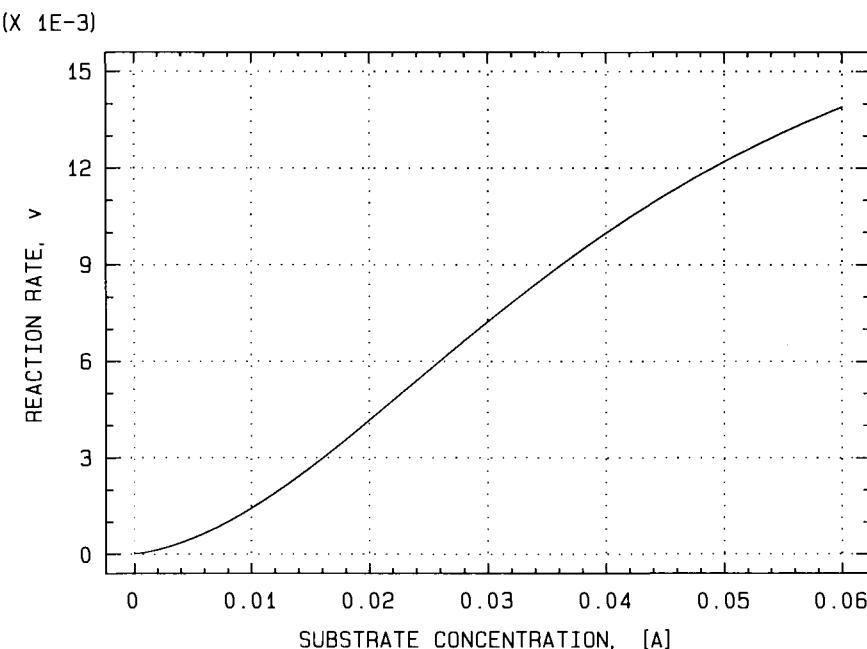
Such equations, empirical or not, also apply in more complex kinetics as well. Reactions with rates expressed by equations in the form of Equation 3.15 exhibit *cooperativity* in many cases. This cooperativity often is explained by use of allosteric mechanisms (Mahler and Cordes, 1971; Laidler and Bunting, 1973; NC-IUB, 1982). The limiting velocity, from Equation 3.15 when  $n = m$ , is

$$V = \frac{\alpha_n}{\beta_n}, \quad (3.16)$$

A plot of  $\ln[v/(V - v)]$  versus  $\ln[A]$  is called a Hill plot, and the slope of this curve is called the Hill coefficient,  $h$ . A system exhibits positive cooperativity when  $h > 1.0$ , noncooperativity when  $h = 1.0$ , and negative cooperativity when  $h < 1.0$ . *Sigmoid kinetics* exist when the  $v$  versus  $[A]$  curve goes through an inflection from concave up to concave down (from positive to negative second derivative). As Laidler and Bunting (1973) showed for  $n = m = 2$ , sigmoid kinetics exist if  $(1/\beta_1) > (\alpha_1/\alpha_2)$ , a special case being when  $\alpha_1 = \beta_1 = 0$ . The actual mechanism usually has little importance in bioreactor design, except possibly in establishment of a correct rate expression. The rate equation, however, is important. The rate curve is significantly different from the hyperbolic Michaelis–Menten curve, as Figure 3.5 shows.

The special case of negative cooperativity fitting the form of Equation 3.15, with  $n = 1$  and  $m = 2$  ascribed to the mechanism in which the enzyme binds with substrate at the active site and more loosely at a second site thereby forming an inactive ternary complex, is most often called *substrate inhibition*. This case usually is expressed in a form similar to the Michaelis–Menten equation (NC-IUB, 1982), namely,

$$v = \frac{V'[A]}{K'_{mA} + [A] + \left(\frac{[A]^2}{K_{iA}}\right)}, \quad (3.17)$$



**FIGURE 3.5** Rate curve for sigmoid enzyme kinetics, single substrate.

where  $K'_{mA}$  = constant specific for this equation (analogous to Michaelis constant), M;

$V'$  = constant,  $M \cdot s^{-1}$ ;

$K_{iA}$  = substrate inhibition constant, M.

Figure 3.6 shows the general form of the rate curve for substrate inhibition.

For bimolecular reactions, Equation 3.6 often is rearranged as

$$v = \frac{V[A][B]}{K_{iA}K_{mB} + K_{mB}[A] + K_{mA}[B] + [A][B]}, \quad (3.18)$$

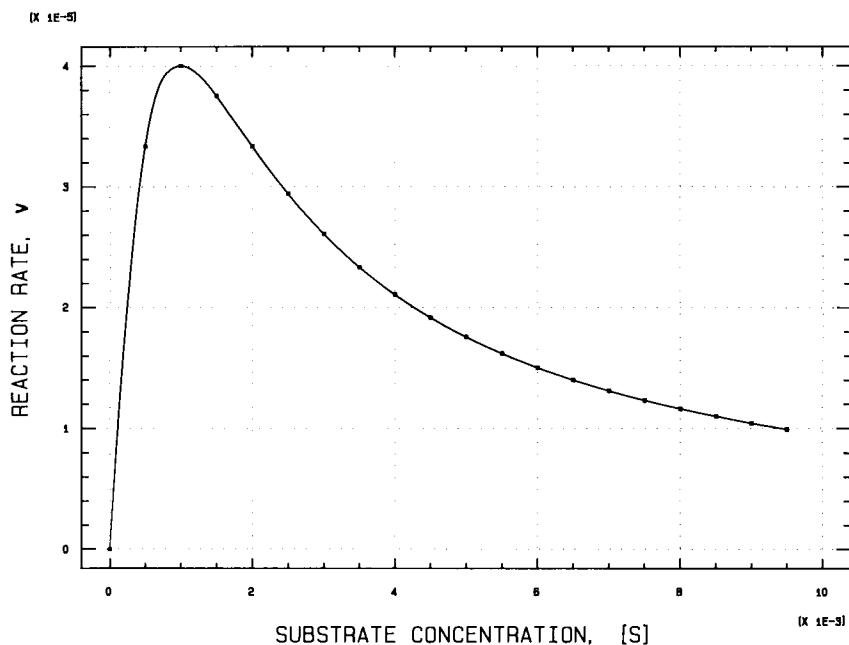
where  $K_{iA}$  = inhibition constant for A ( $= k_B/k_{AB}$ ), M;

$K_{mB}$  = apparent Michaelis constant for B ( $= k_0k_B$ ), M;

$K_{mA}$  = apparent Michaelis constant for A ( $= k_0k_A$ ), M.

The terms  $K_{iA}K_{mB}$  and  $K_{iB}K_{mA}$  are equivalent, where  $K_{iB}$  is the inhibition constant for B ( $= k_A/k_{AB}$ ) and  $K_{mA}$  is the apparent Michaelis constant for A ( $= k_0k_A$ ). If Equation 3.18 is integrated for A with respect to  $t$  for the simple irreversible reaction





**FIGURE 3.6** Rate curve for enzymatic reaction with substrate inhibition.

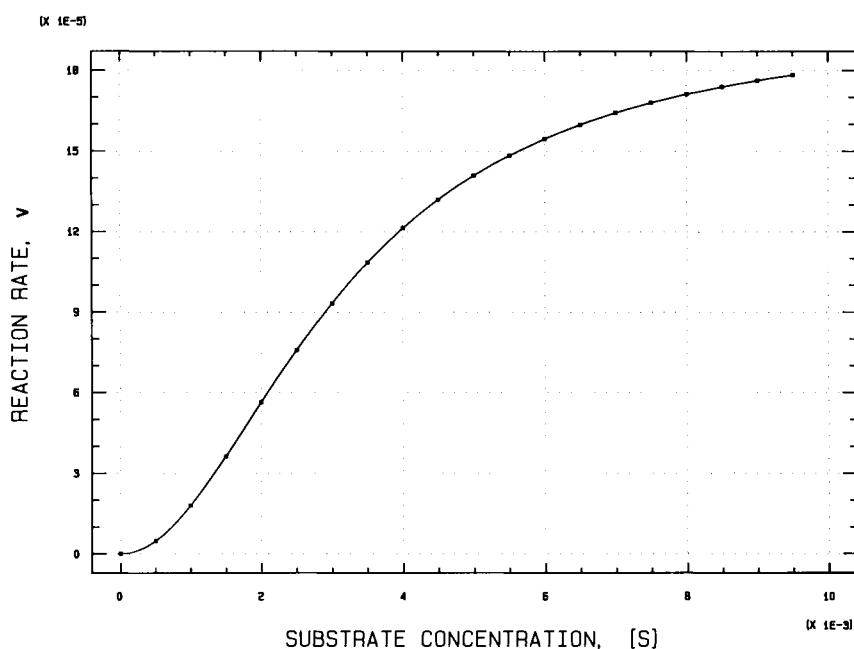
the result is

$$\begin{aligned} & \frac{K_{iA}K_{mB}}{\{[B]_0 - [A]_0\}} \ln \frac{[A]_0([B]_0 - [A]_0 + [A])}{[B]_0[A]} + K_{mB} \ln \frac{[B]_0}{[B]_0 - [A]_0 + [A]} \\ & + K_{mA} \ln \frac{[A]_0}{[A]} + [A]_0 - [A] = Vt. \end{aligned} \quad (3.20)$$

The result is indeterminant when  $[A]_0 = [B]_0$ . In this case the integral form of Equation 3.18 is as follows:

$$K_{iA}K_{mB} \left\{ \frac{1}{[A]} - \frac{1}{[A]_0} \right\} + (K_{mB} + K_{mA}) \ln \frac{[A]_0}{[A]} + [A]_0 - [A] = Vt. \quad (3.21)$$

Equation 3.21 also applies to second-order kinetics for a single substrate when the reaction is  $A + A = \text{products}$  rather than  $A + B = \text{products}$ . The rate curve for either of these cases follows the form shown in Figure 3.7. This rate model also exhibits sigmoid form. There are two general classifications of second-order mechanisms: sequential and ping-pong (Cleland, 1970; Laidler and Bunting, 1973). In a *sequential* reaction, all reactants must combine with the enzyme before the reaction can take place and any products be released.



**FIGURE 3.7** General form of the rate curve for second-order irreversible enzyme kinetics.

In a *ping-pong* type reaction, one or more products are released before all substrate molecules have been added to the enzyme. Equation 3.18 describes kinetics for the sequential mechanism. There is no constant term in the denominator of Equation 3.18 for the ping-pong mechanism. So far as reactor modeling is concerned, the latter case can be considered a special case of Equation 3.18 in which  $K_{iA} = 0$ . Other types of models apply to more complex inhibition and are discussed after factors affecting all models are considered.

### EFFECT OF TEMPERATURE ON ENZYMATIC REACTIONS

Enzyme-catalyzed reactions have a major advantage in that they generally occur at relatively low temperatures compared to those required for other industrial reactions. Attempts to increase reaction rates by increasing temperature are limited by some of the same thermodynamic properties that lead the enzymes to be so effective at low temperatures; however, choice of setting and means of control of reaction temperature are important aspects of bioreactor design. In many cases, modeling temperature effects can be quite complex. Not only are elementary reaction rate constants all functions of temperature,

but Michaelis constants, inhibition constants, and Dalziel constants also are all temperature dependent. Moreover, basic enzyme structure and, consequently, enzyme activity are strongly affected by temperature. A further complication in many cases is the effect of temperature on the nature of substrate, product, inhibitors, activators, coenzymes, membranes and the medium itself. Ultimately, the model must allow for temperature effects on all these parameters.

In practice, however, separating all these effects in models generally is not necessary. The most commonly used models lump all temperature effects as characteristics of the enzymes. But separating reversible and irreversible temperature effects is essential. A lower limit to practical conversions is the freezing point of the medium for reactions normally occurring in aqueous solutions. As temperature increases, catalyzed reaction rates usually increase in a manner that can be closely matched by use of a simple Arrhenius equation. The complications arise as temperatures increase to the point where protein structures in the enzyme molecule are affected. Heat effects may be results of reversible or irreversible denaturations. Reversible denaturations are treated either as instantaneous or time dependent. The most common approach is to treat the reversible effects as though they occur instantaneously, with equilibrium limits being determined by use of the van't Hoff relationships. If the enzyme is considered to exist in either normal, *n*, or denatured, *d*, form, the equilibrium relationship for reversible denaturation is

$$K_1 = \frac{[E]_d}{[E]_n} \quad (3.22)$$

Then, as all the enzyme is assumed to be in one or the other form,

$$[E]_n + [E]_d = [E]_0. \quad (3.23)$$

Combining Equations 3.22 and 3.23 yields

$$[E]_n = \frac{[E]_0}{1 + K_1}. \quad (3.24)$$

The temperature relationship for  $K_1$  is most conveniently determined from thermodynamic properties measured at 25 °C, because they are the most commonly available in the literature. The simplification of assuming that  $\Delta C_p$  for the denaturation reaction is equal to zero and thus that  $\Delta H^\circ$  is constant is not justified, because  $\Delta C_p$  for denaturation of proteins is large (Edsall and Gutfreund, 1983; Pfeil, 1986). The value of  $K_1$  can be determined directly from Equation 3.25, if values of  $\Delta G^\circ$  are available for the unfolding reaction at the desired temperature:

$$K_1 = \exp\left(\frac{-\Delta G_1^\circ}{RT}\right). \quad (3.25)$$

Usually,  $\Delta G^\circ$  for the unfolding process is available at 25 °C as are  $\Delta H^\circ$  and  $\Delta C_p$ . Thus  $K_1$  can be obtained for a temperature of 25 °C (298 K):

$$R \ln K_T = \frac{-\Delta G_{298}^\circ}{T} - \frac{\Delta H_{298}^\circ - (298 \text{ K})\Delta C_p}{T} + \Delta C_p \ln \frac{T}{298}. \quad (3.26)$$

The van't Hoff relationship in Equation 3.26 (from integration of Equation 2.35 or 2.36) can be used for determining  $K_1$  at other temperatures. Of course, if thermodynamic values are available for a reference temperature other than 298 K, Equation 3.26 may be revised easily for that reference temperature.

The basic temperature dependence of the catalyzed reaction at a normal active site at constant pressure may be expressed by using the Arrhenius form of Eyring's absolute reaction rate form (Johnson, Eyring, and Stover, 1974). The appropriate specific reaction rate form for the Arrhenius equation is

$$k = A \exp\left(\frac{-\mu}{RT}\right), \quad (3.27)$$

where  $k$  = specific reaction rate constant;

$A$  = frequency factor;

$\mu$  = activation energy, J/mol.

The units of  $k$  and  $A$  depend on the order of the reaction. The Eyring absolute reaction rate equation presents  $k$  for first-order breakdown of the activated complex as a more complex function of pressure and temperature:

$$k = \left(\frac{kT}{h}\right) \exp\left(\frac{-\Delta H^\ddagger}{RT}\right) \exp\left(\frac{\Delta S^\ddagger}{R}\right), \quad (3.28)$$

where  $k$  = Boltzmann constant, J/K;

$h$  = Planck constant, J·s;

$\Delta H^\ddagger$  = enthalpy of activation, J/mol;

$\Delta S^\ddagger$  = entropy of activation, J/(mol·K).

Equation 3.28 is useful in modeling the combined effects of temperature and pressure on all the elementary reactions involving substrate, enzyme, and combinations (Johnson, Eyring, and Stover, 1974) because

$$\Delta H^\ddagger = \Delta H_0^\ddagger + P \Delta V^\ddagger, \quad (3.29)$$

where  $\Delta H_0^\ddagger$  = enthalpy of activation at zero pressure, J/mol;

$\Delta V^\ddagger$  = volume change of activation, m<sup>3</sup>/mol.

In most cases elevated pressures have appreciable effects on rates of enzyme-catalyzed reactions. These effects must be determined experimentally, but Equations 3.28 and 3.29 are useful in interpreting such experimental data (Johnson, Eyring, and Stover, 1974).

Reversible temperature effects on substrate binding, as reflected in effects on the Michaelis constant, can be modeled with van't Hoff type equations with constant standard-state enthalpy of binding: Equation 3.26, with  $\Delta C_p = 0$  (Johnson, Eyring, and Stover, 1974).

The maximum velocity for Michaelis-Menten kinetics without inhibition and with excess substrate, cofactors, and activators is the velocity used in a large percentage of enzyme reactor designs. The effect of temperature on this velocity can be simulated closely with a simplified relationship ascribing all reversible temperature effects to the enzyme itself:

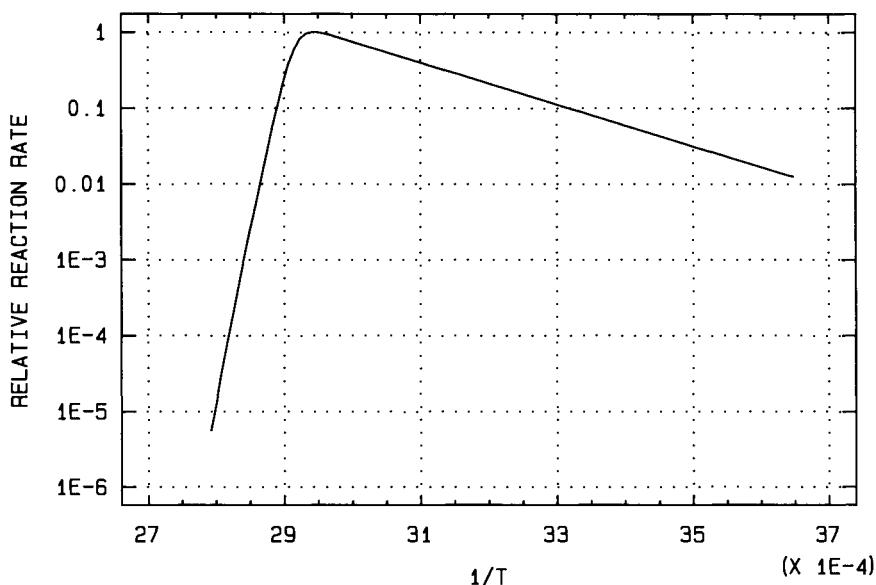
$$V = cT[E]_n \exp\left(\frac{-\Delta H^\ddagger}{RT}\right), \quad (3.30)$$

where  $c$  = a constant,  $\text{K}^{-1}\text{s}^{-1}$ .

Johnson, Eyring, and Stover (1974) showed that

$$\Delta H^\ddagger = \mu - RT. \quad (3.31)$$

The value of  $RT$  is usually smaller than the error in determination of  $\mu$  or  $\Delta H^\ddagger$ . Therefore  $\Delta H^\ddagger$  is generally determined as a constant from the slope of the Arrhenius plot in the region where no appreciable portion of the enzyme is denatured. Thus  $\mu$  and  $\Delta H^\ddagger$  are customarily interchangeable. The type of curve obtained from a plot of Equation 3.30 is common to most enzyme-catalyzed reaction rates and to rates of processes—including multiplication—in organized living systems and organisms. Figure 3.8 shows a typical plot of the relative maximum rate for peptide hydrolysis by trypsin obtained by use of thermodynamic values from different sources (and thus not exactly representative of any particular trypsin preparation). The quantities used for this plot are: for  $\Delta H^\ddagger = 50,200 \text{ J/mol}$  (Netter, 1959); for the reversible denaturation reaction,  $\Delta H_{298}^\circ = 259.4 \text{ kJ/mol}$ ,  $\Delta G_{298}^\circ = 67.0 \text{ kJ/mol}$ , and  $\Delta C_p = 11.9 \text{ kJ/(mol-K)}$  (Pfeil, 1986). The unique characteristic of this type of plot is the precipitous drop in activity after the maximum. In Figure 3.8, the relative activity drops to 10% of the maximum at a temperature of 73 °C. Thus operations with this enzyme are essentially limited to temperatures below 66 °C. Actually, the limit is generally lower than the maximum determined from such a plot for two reasons. First, the gain from increased temperature is less than that which would be obtained from uncatalyzed reaction or reaction with nonenzymatic catalysis. This result is a consequence of the low activation energy of enzyme-catalyzed reactions. Second, irreversible enzyme-denaturation re-



**FIGURE 3.8** Effect of absolute temperature,  $T$  (K), on relative activity of trypsin having the thermodynamic properties listed in the text. Maximum activity occurs at a temperature of 66 °C.

actions are more likely to occur at elevated temperatures. For one-time utilization of enzymes in rapid reactions, however, this characteristic probably would be of no concern.

Denaturation of enzymes, both reversible and irreversible, is sensitive to the presence of denaturing agents, pH, and other environmental conditions. Enzymes can be stabilized by immobilization, cross-linking, and adjustment of environmental conditions such as pH, redox potential, phosphate concentration, and ionic strength. For expensive enzyme systems, extensive laboratory development of optimal operating conditions and systems can easily be justified. In general, denaturation resulting in deactivation or other types of reactions having the same result are treated as first-order reactions. Ordinarily thermal deactivation reactions have high enthalpies of activation (Johnson, Eyring, and Stover, 1974), so the rates are greatly temperature dependent.

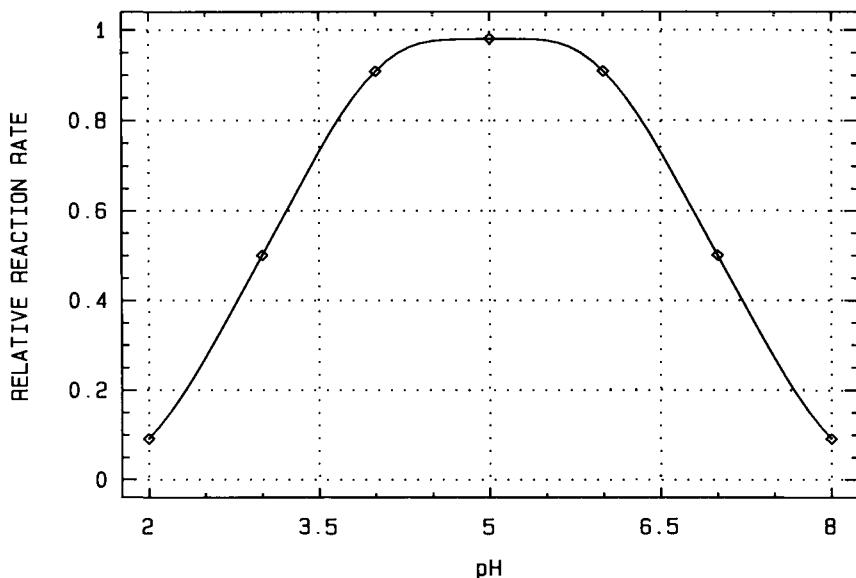
### EFFECT OF PH ON ENZYME-CATALYZED REACTIONS

Control of pH is essential for most enzyme-catalyzed reactions. There is generally an optimal pH, but, as suggested, its magnitude depends on environmental conditions. A plot of rate versus pH may show a sharp decline on either

side of the optimum. The effects of pH on the enzyme may be reversible or irreversible, and the nature of these effects can be determined only by experimentation. The direct effects can be on the enzyme, substrate, cofactors, or other relevant species in the system. In a few cases, specific rate constants of one or more elementary reactions are directly affected by single protonation–deprotonation reactions. Then the reaction rate is a relatively simple function of hydrogen ion concentration:

$$v = \frac{v_m}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}} \quad (3.32)$$

where  $K_1$  and  $K_2$  are usually acid dissociation constants with units of mol/L, and  $v_m$  is the reaction rate at the optimal pH, which lies halfway in between  $pK_1$  and  $pK_2$ . Figure 3.9 illustrates the type of relative rate curve expected for reactions following Equation 3.32. The magnitudes of  $pK_1$  and  $pK_2$  are 3.0 and 7.0, respectively. The peak activity is at the midpoint between the two: a pH of 5.0. The activity is about half the maximum at each of the  $pK$  values. If the two  $pK$  values were closer together, the peak would be sharper and closer control would be necessary. In reactor design the goal for a single reaction is to operate as close to the optimum as is economically feasible. If several



**FIGURE 3.9** pH dependence of an enzymatic reaction following Equation 3.32, with  $pK_1 = 3.0$  and  $pK_2 = 7.0$ .

different reactions occur in the same reactor, optimization becomes a bit more difficult. It is important in all cases.

## ENZYME INHIBITION

### Types of Inhibitors

Inhibitors are substances normally found in a naturally occurring reaction system, foreign-substances contaminants, or unexpected reaction retardants. As discussed previously, substrate or products may also be inhibitors. Degree of inhibition may depend on pH or the presence of other substances in the reaction mixture. The most common inhibitors that must be dealt with are reaction substrates and/or products.

### Reversibility of Inhibition

Inhibition of enzymatic reactions may be reversible, irreversible, or somewhere between the two extremes. Even if inhibition is reversible, the rate of reversal may be so slow that inhibition must still be considered essentially irreversible. Either reversible or irreversible inhibition may be so slow that their effects must be considered as rate limited. Because absolutely irreversible inhibition results in permanently inactive enzymes, the process is also called *deactivation*. The term is not reserved for irreversible inactivation, however.

### Time-dependent Deactivation

Deactivation may result from direct reaction of enzyme with inhibitor or from secondary effects of inhibitor in accelerating normal thermal deactivation. More than one reaction may be involved, and the deactivation process—thermal or otherwise—may involve production of more than one form of enzyme in a series of reactions, especially when enzyme stabilizing factors are present (Henley and Sadana, 1984; Sadana and Henley, 1987). With simple first-order irreversible deactivation the reaction is



The species  $E_d$  is considered to be completely inactive, so the active enzyme concentration available for catalysis elementary reactions is

$$[E]_t = [E]_0 \exp(-k_d t), \quad (3.34)$$

where  $[E]_t$  = active enzyme concentration at time,  $t$ , M;

$k_d$  = specific reaction rate constant for deactivation reaction,  $s^{-1}$ ;

$t$  = time, s.

In many instances, especially in the presence of stabilizing agents or conditions, deactivation is incomplete and activity levels out to a constant level (at least within the time frame of the major deactivation reaction). This phenomenon can be explained by use of two different models. In one, deactivation is equilibrium limited so that the final mixture of active and inactive enzyme moieties has a diminished activity, which is determined by the equilibrium content of active species. In the other case (Henley and Sadana, 1984; Sadana and Henley, 1987) the enzyme species produced by one or more steps have activities that are different (higher or lower) from that of the native form. The higher activities result from some initial stabilization reactions. For example, consider the conversion,



where  $E_1$  is a form of the enzyme that has an activity differing from that of  $E$ , the native enzyme. The reaction is still usually first order, and the concentration of form  $E$  can still be determined by using Equation 3.34. The total activity, however, is the sum of activities contributed by the native form,  $E$ , and the second form,  $E_1$ . The limiting activity is that of form  $E_1$ .

### Equilibrium Inhibition Models for Michaelis–Menten Kinetics

Several standard mechanisms utilized to explain the effects of reversible inhibition are based on Michaelis–Menten kinetics and can be modeled by using modifications of Equation 3.6 (NC–IUB, 1982) with linear functions of  $I$ , or inhibitor concentration. The modifications are as follows for the four major categories.

- *Competitive inhibition*

$$\frac{1}{k_A^{\text{app}}} = \frac{1}{k_A} \left( 1 + \frac{I}{K_{ic}} \right) \quad (3.36)$$

- *Uncompetitive inhibition*

$$\frac{1}{k_o^{\text{app}}} = \frac{1}{k_o} \left( 1 + \frac{I}{K_{iu}} \right) \quad (3.37)$$

- *Mixed inhibition* both equations 3.6 and 3.37 apply
- *Pure noncompetitive inhibition* mixed inhibition, with  $K_{ic} = K_{iu}$

where  $k_A^{\text{app}} = \text{apparent value of } k_A, \text{ L/(mol}\cdot\text{s);}$

$I = \text{inhibitor concentration, mol/L;}$

$K_{ic} = \text{competitive inhibition constant for } I, \text{ mol/L;}$

$K_{iu} = \text{uncompetitive inhibition constant for } I, \text{ mol/L;}$

$k_o^{\text{app}} = \text{apparent value of } k_o, \text{ s}^{-1}.$

The overall rate equation(s) become complex when all these factors are included. Only in the simpler cases can accurate integrations be carried out without the use of numerical integration.

Most initial rate equations fall into one standard category or another. The models for these categories are conveniently stated in simpler standard forms analogous to the Michaelis–Menten equation or its reciprocal, the Lineweaver–Burk form.

- *For uninhibited reaction*

$$\text{Standard: } v = \frac{Va}{a + K_{ma}} \quad (3.2)$$

$$\text{Reciprocal: } \frac{1}{v} = \frac{K_{ma}}{V} \left( \frac{1}{a} \right) + \frac{1}{V} \quad (3.38)$$

- *For competitive inhibition*

$$\text{Standard: } v = \frac{Va}{a + K_{ma} \left( 1 + \frac{i}{K_{ic}} \right)} \quad (3.39)$$

$$\text{Reciprocal: } \frac{1}{v} = \frac{K_{ma}}{V} \left( 1 + \frac{i}{K_{ic}} \right) \left( \frac{1}{a} \right) + \frac{1}{V} \quad (3.40)$$

- *For uncompetitive inhibition*

$$\text{Standard: } v = \frac{Va}{K_{ma} + a \left( 1 + \frac{i}{K_{iu}} \right)} \quad (3.41)$$

$$\text{Reciprocal: } \frac{1}{v} = \frac{K_{ma}}{V} \left( \frac{1}{a} \right) + \frac{1}{V} \left( 1 + \frac{i}{K_{iu}} \right) \quad (3.42)$$

- For mixed inhibition

$$\text{Standard: } v = \frac{Va}{K_{ma} + a + \frac{iK_{ma}}{K_{ic}} + \frac{ai}{K_{iu}}} \quad (3.43)$$

$$\text{Reciprocal: } \frac{1}{v} = \frac{K_{ma}}{V} \left( 1 + \frac{i}{K_{ic}} \right) \left( \frac{1}{a} \right) + \frac{1}{V} \left( 1 + \frac{i}{K_{iu}} \right) \quad (3.44)$$

where  $a$  = primary substrate concentration;

$i$  = inhibitor concentration.

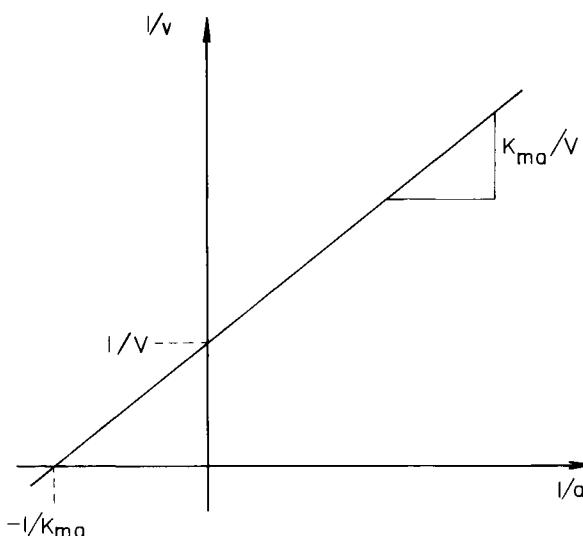
Confusing terminology has arisen for the special case of mixed inhibition, where  $K_{ic} = K_{iu}$ , which traditionally is called *noncompetitive inhibition*. Not only is there confusion between the terms *noncompetitive inhibition* and *uncompetitive inhibition*, but also among the various types of mixed inhibition, which may also have been called *noncompetitive inhibition*. The Nomenclature Committee of the International Union of Biochemistry recommends that this special case of mixed inhibition, where  $K_{ic} = K_{iu}$ , be called *pure noncompetitive inhibition* and that the term *noncompetitive inhibition* not be used at all. In line with these recommendations, the equations for pure noncompetitive inhibition are

$$\text{Standard: } v = \frac{Va}{\left( K_{ma} + a \right) \left( 1 + \frac{i}{K_i} \right)} \quad (3.45)$$

$$\text{Reciprocal: } \frac{1}{v} = \frac{K_{ma} \left( 1 + \frac{i}{K_i} \right)}{V} \left( \frac{1}{a} \right) + \frac{1}{V} \left( 1 + \frac{i}{K_i} \right) \quad (3.46)$$

The reciprocal, or Lineweaver–Burk, equations are useful in determining the type of inhibition involved and the magnitudes of the inhibition constants. In all these reciprocal forms, plots of  $1/v$  versus  $1/a$  or  $a/v$  versus  $a$  at constant values of  $i$  and  $1/v$  versus  $i$  or  $a/v$  versus  $i$  at constant values of  $a$  should give straight lines. Constants are obtained from the slopes and intercepts of these straight-line plots. For example,  $K_{ma}/V$  and  $1/V$  are obtained from a plot of  $1/v$  versus  $1/a$  for the uninhibited reaction shown in Figure 3.10.

There are statistical arguments for minimizing experimental error effects by using plots or regressions of  $a/v$  versus  $a$ . The data should be analyzed for the best relationship to use. Generally, a linear regression analysis is used in



**FIGURE 3.10** Lineweaver-Burk plot for determination of constants  $V$  and  $K_{ma}$  for uninhibited reaction.

preference to a graphic analysis, although being able to look at the graphs always helps. In some cases the maximum rate,  $V$ , may be determined by direct observation. Moreover,  $K_{ma}$  is equal to the substrate concentration giving a rate equal to half the maximum rate. Care should be taken in the use of this simplified analysis to be sure that a true maximum rate is obtained and that the reaction actually is uninhibited. All the reciprocal plot applications are for constant initial enzyme concentrations. The catalytic constant,  $k_o$ , is the slope of a plot of  $V$  versus  $[E]_0$ , according to Equation 3.5 for the uninhibited reaction. Then, as previously discussed,

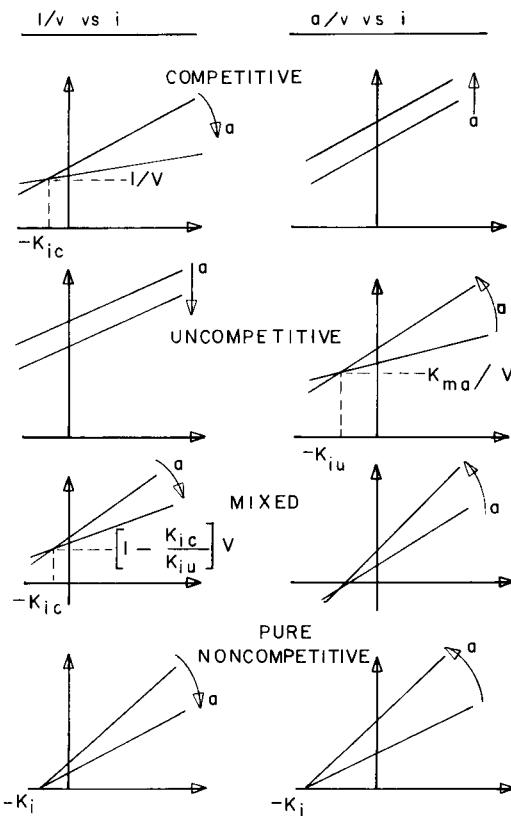
$$\frac{k_o}{k_A} = K_{ma},$$

and

$$k_A = \frac{k_o}{K_{ma}}. \quad (3.47)$$

Some types of reciprocal plots used for analysis of the various inhibition categories are illustrated in Figure 3.11.

As a special case of uncompetitive inhibition, substrate inhibition must be



**FIGURE 3.11** Plots of reciprocal inhibition equations with inhibitor concentration,  $i$ , as the independent variable and substrate concentration,  $a$ , as a parameter.

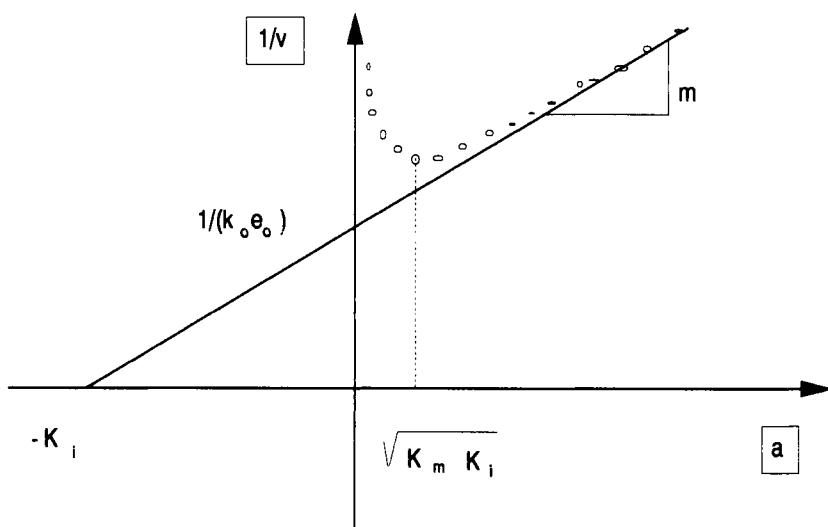
treated in a slightly different manner to obtain constants. For substrate inhibition—because  $a$  and  $i$  are now the same—Equation 3.42 becomes

$$\frac{1}{v} = \frac{K_{ma}}{k_o e_o} \left( \frac{1}{a} \right) + \frac{a}{K_i k_o e_o} + \frac{1}{k_o e_o}, \quad (3.48)$$

where  $e_o$  = total enzyme concentration.

Figure 3.12 shows the plot used for determining constants for Equation 3.48. The constants are obtained from an extension of a tangent to the curve at high substrate concentration, where the term  $1/a$  becomes increasingly small. The actual maximum reaction rate (minimum point in Figure 3.12) is attained at the point  $a = \sqrt{K_{ma} K_i}$ , so  $k_o e_o$  is used instead of  $V$ .

The analysis of inhibition models according to Michaelis–Menten kinetics also may be applied conveniently to cell growth inhibition, because the sim-



**FIGURE 3.12** Typical plot for determinating constants for substrate inhibition of an enzymatic reaction. Slope of the tangent is  $m = 1/(K_i k_o)$ . Adapted from M. Dixon and E. C. Webb, *Enzymes*, 3rd. ed., Longman Group, Ltd., [1979] p. 129. (Reproduced with permission from the author and publisher.)

plified equation forms presented here are the same as those derived from the Monod equation. This result naturally follows, because the Monod equation presents a specific growth rate,  $\mu$ , as a Michaelis-Menten function of cell substrate concentration. However, cell multiplication may require more complex models and different treatment, just as some complex enzymatic reactions do.

## KINETICS OF POLYMER HYDROLYSIS

An important application of bioreactor design is hydrolysis of polymeric materials, such as starches, proteins, and cellulose, in which the polymers may be only slightly soluble. Enzymatic attack usually takes place at multiple sites, and enzyme preparation may consist of several different enzymes with different functions. Additionally, different forms of the polymers existing even in the same molecules may exhibit dissimilar kinetics. These kinetics may be further complicated by product inhibition. One example of a system involving all these factors is the enzymatic hydrolysis of cellulose.

In natural and waste materials cellulose usually occurs along with other materials, such as lignins, hemicellulose, and the like. Cellulose is an unbranched polymer of glucose monomers linked by  $\beta$ -1,4-glucosidic bonds. These polymers occur in long bundles of microfibrils that can contain ordered crystalline regions and amorphous regions. As discussed by Wald *et al.* (1984), the

cellulase enzyme systems used to hydrolyze cellulose may contain varying proportions of three main enzyme groups:

1. an endoglucanase,  $\beta$ -1,4-glucanohydrolase;
2. an exoglucanase,  $\beta$ -1,4-glucan cellobiohydrolase; and
3.  $\beta$ -glucosidase.

The cellulose molecules shrink with reaction. Humphrey *et al.* (1977) developed a *shrinking-site model*, in which they assumed the rate of cellulose hydrolysis to be proportional to the number of sites on the substrate and the fraction of these sites occupied by enzyme molecules. These rates were then reduced by product inhibition. Holtzapple *et al.* (1984) then refined the shrinking-site model to derive a *HCH-1 model*, which accounted for active and inactive cellulose forms. Wald *et al.* (1984) and Ryu *et al.* (1982) took a different approach by assuming that kinetics differ for the crystalline and amorphous cellulose forms. Mandenius *et al.* (1988) reviewed various models and showed that the Wald model fit their data well. This model, along with the Gong *et al.* model, as reported by Mandenius *et al.*, has two distinct series of reactions:



where  $G_{2C}$  = crystalline cellulose;  
 $G_{2A}$  = amorphous cellulose;  
 $G_2$  = cellobiose;  
 $G_1$  = glucose.

The rate equations for cellulose disappearance and cellobiose appearance according to those pathways then are

$$-\frac{dG_{2C}}{dt} = k_4 E_{aC}, \quad (3.49)$$

and

$$-\frac{dG_{2A}}{dt} = k_5 E_{aA} \frac{1}{1 + K_{i,4} G_2}, \quad (3.50)$$

where  $k_4, k_5$  = rate constants;

$E_{aC}$  = concentration of enzyme adsorbed on crystalline cellulose;

$E_{aA}$  = concentration of enzyme adsorbed on amorphous cellulose;

$K_{i,4}$  = cellobiose inhibition constant;

$G_2$  = cellobiose concentration.

Bailey and Ollis (1986) discuss the Ryu *et al.* (1982) model.

# Chapter 4

---

## Mass-Transfer Fundamentals

### MASS-TRANSFER LIMITATIONS

Mass-transfer limitations must be considered as possibilities in determining kinetic relationships for any reactor design. Significant categories in bioreactor design are (1) intraphase diffusion in the medium, (2) interphase mass transfer at gas–liquid interfaces, (3) mass transfer through membranes, (4) mass transfer through porous structures, (5) interphase transfer at liquid–liquid interfaces, and (6) interphase transfer at solid–liquid interfaces. The usual practice is to make simplifying assumptions for mass-transfer models because of the difficulties in determining constants for the more complex models that probably best depict the actual systems. More sophisticated models undoubtedly will be developed and used in design, but they are now generally limited to use in research and most are not covered in detail here.

Mass-transfer rates in bioreactors are significant because of their effects on some chemical reaction rates, either in transformations by enzymes or by living cells. The latter effects are important either in the rate of cell growth or in the transformation of substrate to products. In many cases mass-transfer limitations are negligible, but that must be confirmed for informed reactor design. Mass transfer may not be a limit in a laboratory-sized reactor, but it may become a significant limit in larger production reactors. Immobilized enzymes and immobilized cells quite often have mass-transfer rate limitations; or at least mass-transfer resistance must be considered as a significant overall rate determinant. Microbial flocs and mycelial mats and balls also have significant mass-transfer rate limitations. These special cases are treated in more detail as specific subjects in later chapters.

### INTRAPHASE MASS TRANSFER

Intraphase mass-transfer rates for molecular diffusion can be modeled by using Fick's first law of diffusion with nomenclature as outlined by Treybal

(1980). Fick's first law for diffusion of component A in a mixture of A and B is

$$J_{Az} = -D_{AB} \frac{\partial C_A}{\partial z}, \quad (4.1)$$

where  $J_{Az}$  = molar flux of A in the  $z$  direction, relative to bulk flow, mol/(cm<sup>2</sup>·s);

$D_{AB}$  = diffusivity (or diffusion coefficient) of A in medium B, cm<sup>2</sup>/s;

$C_A$  = concentration of A, mol/cm<sup>3</sup>;

$z$  = distance in  $z$  direction, cm.

In biological reactors, medium B is the growth medium or the liquid within a semisolid portion of the growth system. As a first approximation for aqueous liquid, medium B may be assumed to be water with a correction applied for the viscosity of the actual medium. A widely used equation for approximation of  $D_{AB}$  for a dilute solution of A in solvent B for nonelectrolytes is the Wilke–Chang equation (Reid, Prausnitz, and Poling, 1987), which is dimensionally inconsistent:

$$D_{AB} = \frac{7.4 \times 10^{-8}(\phi M_B)^{1/2} T}{\eta_B V_A^{0.6}}, \quad (4.2)$$

where  $\phi$  = association factor of solvent B, dimensionless;

$M_B$  = molecular weight of solvent B, g/mol;

$T$  = temperature, K;

$\eta_B$  = viscosity of solvent B, cp;

$V_A$  = solute A molar volume at normal boiling point, cm<sup>3</sup>/mol.

The magnitude of  $\phi$  is taken as 2.6 for water as a solvent (Treybal, 1980). Values of  $V_A$  may be obtained from Reid, Prausnitz, and Poling (1987). For transport systems in which there is a net flow of material, a bulk transport term must be considered. The net flux of component A in a binary fluid mixture of A and B then is

$$N_{Az} = J_{Az} + u_{mz}C_A, \quad (4.3)$$

where  $N_{Az}$  = molar flux of A in the  $z$  direction with respect to fixed coordinates, mol/(cm<sup>2</sup>·s);

$u_{mz}$  = net molar velocity of mixture in the  $z$  direction, cm/s.

In the case of equimolar counterdiffusion,  $u_{mz} = 0$  and  $N_{Az} = J_{Az}$ .

The use of pure water as the solvent—even with the viscosity corrected to that of the medium—in the Wilke–Chang equation gives only an approximation

for the upper limit to mass-transfer rates controlled by molecular diffusion in aqueous media, but at least the use of rate equations that contain mass-transfer resistances gives a starting point for the correct forms of equations along with limits for constants. However, actual constants must be determined by experiment in most cases. Refinements can be made to correct for electrolyte concentrations by evaluating diffusion coefficients for electrolytes as diffusing or nondiffusing components of the medium, as outlined by Reid, Prausnitz, and Poling (1987).

In semisolid media, such as gels or even flocs of cells or mat supports, effective diffusion coefficients may be used. In such cases the semisolid medium is usually treated as though it were continuous. Effective diffusivities must be determined by analysis of experimental data.

## GAS-LIQUID MASS TRANSFER

The most widely studied mass-transfer limitation of biological systems is that involving transport of oxygen from the gas phase to the reacting systems or cells. In most cases oxygen is obtained from air, but this source is not a necessary limitation. Mathematical treatment of gas-liquid transport in bioreactors is almost universally handled by assumption of a two-film resistance and use of  $k$ -type film coefficients. With further simplification, local overall  $K$ -type coefficients may be used, or a single  $k$ -type film coefficient may be used with the assumption that most of the resistance to mass transfer is in either the gas or the liquid film at the gas-liquid interface. Treybal (1980) discussed the relations among the constants and resistance models.

For transport of a component A from gas to liquid growth media, the flux,  $N_A$ , may be expressed in terms of a concentration-related driving force multiplied by a constant. At steady state the flux leaving through the gas film must equal that entering through the liquid film, so  $N_A$  determined from any of the following equations must be the same:

$$N_A = k_y(y_A - y_{A,i}); \quad (4.4)$$

$$N_A = k_c(c_{A,G} - c_{A,i,G}); \quad (4.5)$$

$$N_A = k_x(x_{A,i} - x_A); \quad (4.6)$$

$$N_A = k_L(c_{A,i,L} - c_{A,L}); \quad (4.7)$$

where  $k_y$  = molar gas mass-transfer coefficient, mol/(cm<sup>2</sup>·s);

$k_c$  = gas concentration mass-transfer coefficient, cm/s;

$k_x$  = molar liquid-phase mass-transfer coefficient, mol/(cm<sup>2</sup>·s);

$k_L$  = liquid concentration mass-transfer coefficient, cm/s;

$y_A$  = mole fraction of A in bulk gas phase, mol/cm<sup>3</sup>;

- $y_{A,i}$  = mole fraction of A in gas phase at gas–liquid interface;  
 $c_{A,G}$  = concentration of A in bulk gas phase, mol/cm<sup>3</sup>;  
 $c_{A,i,G}$  = concentration of A in gas phase at gas–liquid interface, mol/cm<sup>3</sup>;  
 $c_{A,i,L}$  = concentration of A in liquid phase at gas–liquid interface, mol/cm<sup>3</sup>;  
 $c_{A,L}$  = concentration of A in bulk liquid phase, mol/cm<sup>3</sup>;  
 $x_{A,i}$  = mole fraction of A in liquid phase at gas–liquid interface;  
 $x_A$  = mole fraction of A in bulk liquid phase.

The right-hand sides of Equations 4.5 and 4.8 can be equated:

$$k_L(c_{A,i,L} - c_{A,L}) = k_c(c_{A,G} - c_{A,i,G}). \quad (4.8)$$

The usual assumption for steady-state transport through an interface is that the liquid and gas are at equilibrium at the interface. Because oxygen is only slightly soluble in water, the magnitude of  $c_{A,i,L}$  must be extremely low at best for oxygen transport. Analogous liquid-phase mass-transfer coefficients are generally lower than gas-phase mass-transfer coefficients owing to the higher diffusivity of gases. Consequently,  $c_{A,i,G}$  and  $c_{A,G}$  are nearly equal, and  $c_{A,i,L}$  essentially must be equal to the concentration of oxygen in the aqueous medium that prevails at equilibrium with the bulk gas phase. Thus the oxygen flux is given with little error by

$$N_{O_2} = k_L(c_{O_{2,L}}^* - c_{O_{2,L}}), \quad (4.9)$$

where  $c_{O_{2,L}}^*$  = concentration of oxygen given by Henry's law or related equilibrium equation or data for equilibrium with the bulk gas phase, mol/cm<sup>3</sup>;  
 $c_{O_{2,L}}$  = concentration of O<sub>2</sub> in bulk liquid phase, mol/cm<sup>3</sup>.

For aerobic cultures of cells,  $c_{O_{2,L}}$  should be greater than the critical minimum, which, depending on the type of cells involved, usually is about 5% of the normal equilibrium concentration of oxygen in water equilibrated with air at atmospheric pressure (Forage *et al.*, 1985). Equilibrium concentrations of oxygen may be determined using relationships discussed under Phase Equilibrium Relationships in Chapter 2.

Values of  $k_L$  are difficult to determine because of complications in determining interfacial areas in most experimental systems. Thus magnitudes of  $k_{LA}$ , rather than  $k_L$ , are generally determined in oxygen transport studies. The quantity  $a$  is the interfacial area per unit volume of reaction mixture (cm<sup>-1</sup> or m<sup>-1</sup>). Its magnitude depends on the size of gas bubbles introduced into the system in sparged reactors, the nature and conditions of the liquid medium, pressure, temperature, and degree of mixing. Effects of operating variables are covered for specific types of reactors when oxygen transport limitations

are significant. The coefficient  $k_L$  is most often correlated with system parameters as a part of the Sherwood number,  $\text{Sh}$ , which is defined as

$$\text{Sh} = \frac{k_L L}{D_{AB}}, \quad (4.10)$$

where  $L$  is a characteristic length of the system in cm. The majority of correlations for the Sherwood number involve equations of one of the following forms (Kargi and Moo-Young, 1985):

$$\text{Sh} = a + b\text{Re}^c\text{Sc}^d, \quad (4.11)$$

or

$$\text{Sh} = e\text{Gr}^f\text{Sc}^g, \quad (4.12)$$

where  $a$ ,  $b$ ,  $c$ ,  $d$ ,  $e$ ,  $f$ , and  $g$  are empirical constants;

$\text{Gr}$  = Grashof number, defined as  $\text{Gr}_D$  for diffusion as

$$\text{Gr} = \frac{gL^3\rho \Delta\rho}{\mu^2}; \quad (4.13)$$

$\text{Re}$  = Reynolds number, defined as

$$\text{Re} = \frac{Lu\rho}{\mu}; \quad (4.14)$$

where

$g$  = local acceleration of gravity,  $\text{cm/s}^2$ ;

$L$  = characteristic length of system, cm;

$u$  = fluid velocity,  $\text{cm/s}$ ;

$\rho$  = liquid density,  $\text{g/cm}^3$ ;

$\Delta\rho$  = liquid density – gas density,  $\text{g/cm}^3$ ;

$\mu$  = fluid viscosity,  $\text{g}/(\text{cm}\cdot\text{s})$ ;

$\text{Sc}$  = Schmidt number;

$\text{Sc} = \nu/D_{AB}$ ; (4.15)

$\nu$  = kinematic viscosity,  $\text{cm}^2/\text{s}$ ;

$\nu = \mu/\rho$ . (4.16)

In some cases the product  $\text{Re}\cdot\text{Sc}$  is replaced by its equivalent, the Péclet number,  $\text{Pe}$ :

$$\text{Pe} = \frac{Lu}{D_{AB}}. \quad (4.17)$$

The same assumptions made in modeling oxygen mass transfer to aqueous solutions apply to other relatively insoluble gases, such as methane. Thus the preceding discussion of oxygen utilization rates can be extended to methane utilization rates.

## MASS TRANSFER THROUGH MEMBRANES

Mass transfer through membranes involves transport of molecular species through three resistances: the liquid film on the first side of the membrane, the membrane itself, and the liquid resistance on the second side of the membrane. Physical transport through the membrane may be hydrodynamic if a pressure difference exists across the membrane. Furthermore, molecular transport may be through normal molecular diffusion, Knudsen diffusion, or permeation. In Knudsen diffusion the pore diameter is small enough that wall collisions become important in relation to molecular collisions. In permeation the solute undergoing transfer is dissolved in the membrane and transported in dissolved form to the side of lower chemical potential. Osmotic pressures must be considered in determining limits to transport, and further complications exist in relation to compatibility of membranes with solutes. Moreover, specifically designed membranes may advantageously or disadvantageously attract or exclude specific solutes based on polarity or ionic charge.

Because of all these considerations, design of membrane reactors is system specific. Generalities may be applied for the two liquid film resistances and, in general,  $k_L$  values may be determined by equations in the form of Equation 4.11 or 4.12, depending on the nature of the system and flows. In the simplest system involving transport of a single solute across a planar membrane (or curved surface of large radius)—with no appreciable specific exclusion or electrostatic effects and no net bulk flow across the membrane—the three-resistance model yields

$$N_A = \frac{c_{A,1} - c_{A,2}}{\frac{1}{k_{L,1}} + \frac{z}{D_{AB,eff}} + \frac{1}{k_{L,2}}}, \quad (4.18)$$

where  $c_{A,1}$  = concentration of solute A on high side, mol/cm<sup>3</sup>;

$c_{A,2}$  = concentration of solute A on low side, mol/cm<sup>3</sup>;

$k_{L,1}$  = liquid mass-transfer coefficient on high side, cm/s;

$k_{L,2}$  = liquid mass-transfer coefficient on low side, cm/s;

$D_{AB,eff}$  = effective diffusivity of solute A in membrane, cm<sup>2</sup>/s;

$z$  = thickness of membrane, cm.

Equation 4.18 may be corrected easily for curvature effects. For example, for transport of A across a cylindrical tube  $N_A$  based on outside surface and

transport from the outside bulk concentration,  $c_{A,1}$ , to the inside bulk concentration,  $c_{A,2}$ , with  $r_1$  and  $r_2$  being the tube outside and inside radii, respectively, Equation 4.18 becomes

$$N_A = \frac{c_{A,1} - c_{A,2}}{\frac{1}{k_{L,1}} + \frac{r_1 \ln(r_1/r_2)}{D_{AB,\text{eff}}} + \frac{r_1}{r_2 k_{L,2}}}. \quad (4.19)$$

The two liquid film resistances are affected by dimensions and flow rates. The second term of the denominator in Equation 4.18 or 4.19 is affected only by characteristics of the membrane (and fluid within) and dimensions. The film coefficients are estimated from appropriate empirical equations (generally of the form of Equation 4.11 or 4.12, depending on the system). In an experimental system, then, the magnitude of  $D_{AB,\text{eff}}$  may be estimated and Equation 4.18 or 4.19 used in optimizing dimensions and flow rates. Reaction rates may be determined for values of  $c_{A,2}$  to show whether the mass-transfer or reaction rate is limiting. More sophisticated models must be used if neither is limiting. The same applies if reactions are occurring within the membrane.

## MASS TRANSFER THROUGH POROUS STRUCTURES

As for mass transfer through membranes, effective diffusivities generally are used for mass transfer through porous structures, such as those in immobilized enzyme or cell complexes; in microbial flocs, mats, or other agglomerations; or in porous media. In general, however, the designer must consider the effects of both the chemical reaction (and/or growth) and transport. Effects are system specific.

## INTERPHASE MASS TRANSFER AT LIQUID–LIQUID INTERFACES

Interphase mass transfer at liquid–liquid interfaces is encountered, for example, when liquid hydrocarbons are being transformed by microbial or enzymatic reactions. Usually the reactant hydrocarbon is sufficiently insoluble in the aqueous medium that only the liquid film coefficient on the aqueous side need be considered in determining mass-transfer rates. Thus the considerations are identical to those discussed in relation to relatively insoluble gases, such as oxygen, in gas–liquid mass transfer. Liquid droplet sizes depend on how the insoluble liquid is introduced, the degree of mixing, and the properties of the two liquids. Surfactants, either added to the medium or produced by the reacting system, can significantly affect the transfer either by speeding up or slowing down rate, depending on the system. Usually,  $k_L a$  values are reported for experimental systems, especially for design purposes.

## MASS TRANSFER AT SOLID-LIQUID INTERFACES

With one major difference, the considerations for solid-liquid interfacial transport are the same as those for liquid-liquid interfacial transport. The solid particles are either of essentially constant size or of diminishing size in most cases. If the *solids* are flocs or pellets of immobilized systems or wall coatings, mats, and the like, with reactions occurring within, complex models must be developed to explain global reaction rates. In complex situations it is convenient to look at interfacial mass transfer limitations separately to determine whether they are limiting and, if so, how they may be reduced by changing conditions such as flow velocities, particle sizes, and degree of back-mixing.

## GENERAL MASS-TRANSFER CONSIDERATIONS

In most design situations, the designer evaluates mass-transfer limitations separately from other limitations whenever possible. In many cases minimizing the mass-transfer limitations to the point that only reaction or growth rate limitations need be evaluated is possible. Scale-up difficulties are pronounced in most systems when mass transfer is limiting; so not overlooking the possibilities of such limitations in large systems is important. Some cases for example, reaching the same Reynolds numbers—and consequently the same transport rates—as in smaller systems is not possible. In laboratory systems in which organism or enzyme temperature sensitivity do not seriously limit temperature variation, the designer can, in some cases, determine whether mass transfer may be limiting by determining the effect of temperature variation on the mass transfer rate. The effect is usually much lower than for reaction-limited cases. Mass transfer is generally aided by increases of temperature, but there are notable exceptions. The oxygen transport rate is not greatly affected, because higher temperatures, although they increase the diffusivity of oxygen in water, decrease solubility. At the extreme limit this thermodynamic reduction is sufficient to reduce the solubility of oxygen below the critical minimum for many living organisms.

# Chapter 5

---

## Kinetics of Cellular Multiplication

### NATURE OF CELL GROWTH AND MULTIPLICATION

The cell is the unit of life, and so it naturally is the unit of ultimate consideration in most growth processes in bioreactor design. However, the differences between living and nonliving matter often are debated. Are plasmids or viruses living units? Even if they are, they depend on cells for their own reproduction. Prokaryotic and eukaryotic cells are quite different in many aspects, but in treatment of growth rate kinetics of single-cell suspensions or suspensions of small groups of cells the same simple types of lumped models may be used. More complex cell communities, developed naturally or artificially, require special treatment to develop usable models. Therefore discussions applicable to large groups of unrestrained single-cell members are not applicable to organs, organized fungal or plant cell structures, or artificially constrained cells in pores or immobilization matrices. Thus, except where noted, the following discussions are directed to single-cell suspensions or unrestrained layers.

The primary types of cellular reproduction are binary fission, mycelial growth, and budding. Whatever the means of cellular division, individual cells are able to reproduce themselves in an orderly manner, so that genetic material is (usually) faithfully carried in intact codes for many generations. Thus cells descending from any specific germ line may be treated essentially as duplicates. Cells, however, are products of both genetic makeup and environment. In many systems the environment is continually changing—and being changed by the living cells that it surrounds. Individual cells go through cycles from birth to division, and, as individuals or cultures synchronized in development, their properties must be treated as strict time functions. Fortunately, in the typical large populations encountered in most growth systems, cell properties may be treated as averages and convenient lumped models for homogeneous populations of cells may be used. Further, simple unstructured growth models relating processes to rate of growth of population may be utilized in many instances. In other cases the use of more structured process models of segregated cell systems becomes worthwhile.

Populations of cells made up of single cells and pairs or small groups are called *single-cell cultures*. Of necessity, the word *culture* must pertain to the medium as well as the cell population because of the close interrelationship between the two. The batch culture is the culture that develops through cell multiplication after introduction of a small inoculum of cells into fresh medium and is usually maintained at some consistent operating conditions. In true batch reactors nothing would be introduced or produced from the system during the growth process. By convention, however, gases are fed or produced and various reagents are added to maintain constant pH conditions without changing the description of the process. Most batch cultures of single-cell populations exhibit the same general type of growth curve when the growth medium contains all the essential nutrients, vitamins, minerals, and cofactors required for growth. The general form of a batch curve is shown in Figure 5.1.

The first stage of growth is the *lag phase*—a period of adjustment of the cellular inoculum to its new environment. If the cells in the inoculum are taken from an actively growing culture, the lag phase may be short or nonexistent. As the cells adjust to the new environment, they begin to grow and multiply and reach a state in which the rate of division is directly proportional to the number of cells present. It is called the *logarithmic*, or *exponential*, *phase*. Cell reproduction may proceed rapidly, with doubling times on the order of 20 minutes for some cultures. After a while, a limit is ultimately met for various reasons. For example, an essential nutrient may become depleted, toxic substances may build up to limit growth and even kill cells, or cells may suffer from contact inhibition. As one of the growth-limiting conditions occurs, the growth rate is no longer logarithmic and then reaches a point when the net rate of cell production is zero. In this *stationary phase*, the actual rate of

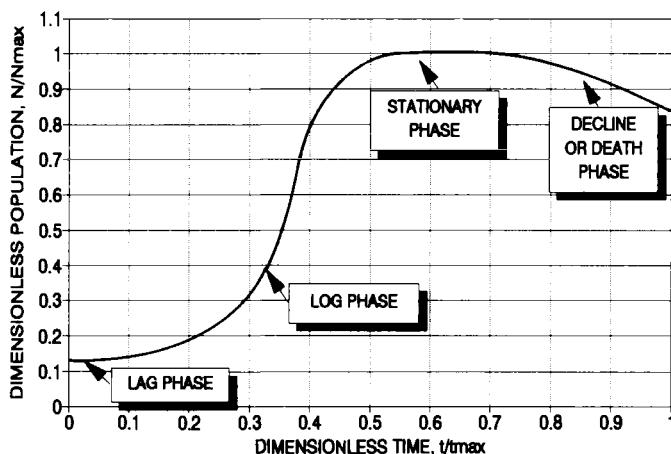


FIGURE 5.1 Typical growth curve for a batch cell culture.

production of new cells is either zero or equal to the rate of death of cells. In most cases the *death, or decline, phase*—in which the cell population begins to decrease—follows the stationary phase.

The logarithmic growth phase is the one most amenable to mathematical treatment. The models developed for this phase are even extended in modified form to other growth phases because of their relative simplicity. In the logarithmic growth phase the general model for average cells is a simple first-order growth rate equation:

$$\frac{dN}{dt} = \mu N, \quad (5.1)$$

where  $N$  = number of cells;

$t$  = time, s;

$\mu$  = specific growth rate,  $s^{-1}$ .

Rearranging Equation 5.1 yields

$$\mu = \frac{1}{N} \frac{dN}{dt} \quad (5.2)$$

or

$$\mu = \frac{d \ln N}{dt}. \quad (5.3)$$

Hence  $\mu$  is called the logarithmic rate constant or the specific rate constant, but  $\mu$  may not actually be constant over a period of rapid growth. If it is, integration of Equation 5.1 yields the familiar logarithmic population equation,

$$\ln \frac{N}{N_0} = \mu t, \quad (5.4)$$

where  $N_0$  = initial cell population at time zero.

Note from Equation 5.4 that the population doubling time—the time for  $N$  to double itself or the average cell multiplication cycle time—can be used to determine the value of  $\mu$  or vice versa. This relationship is expressed in Equation 5.5:

$$\ln(2.0) = \mu t_d \quad (5.5)$$

where  $t_d$  = doubling time.

Thus for a doubling time of 30 min,  $\mu = 0.0231/\text{min}$ , or  $3.85 \times 10^{-4} \text{ s}^{-1}$ . The logarithmic growth rate model is modified for cases in which substrate is limiting.

## MONOD KINETICS

The model most generally used for cell multiplication is the Monod equation. It is used to determine a specific growth rate *constant*, which is a function of substrate concentration. The Monod equation has the same form as the Michaelis–Menten equation, from which it was derived with the assumption that cell growth was limited by some essential enzymatic reaction:

$$\mu = \mu_m \frac{S}{S + K_s}, \quad (5.6)$$

where  $\mu_m$  = maximum specific growth rate constant for growth not limited by substrate concentration,  $\text{s}^{-1}$ ;

$K_s$  = Monod constant, mol/L;

$S$  = concentration of limiting substrate, mol/L.

At substrate concentrations much higher than the value of the Monod constant,  $\mu$  essentially is equal to  $\mu_m$  and growth is zero order with respect to substrate. This is the case for many biological transformations. For example, for glucose as a substrate for *S. cerevisiae*,  $K_s$  can be less than 1 mg/mL (Pirt, 1975). When  $\mu$  is half its maximum value, or  $\mu = \mu_m/2$ ,  $S$  must equal  $K_s$ . One means of evaluating  $K_s$  is to determine the magnitude of  $S$  at half the maximum specific growth rate (as the Michaelis constant is determined for enzymatic reactions). Further decreases of  $S$  below  $K_s$  lead to growth that approaches first order with respect to substrate concentration at low values of  $S$ .

The rate equation for net cell growth may be modified further by incorporating a term for the death rate of cells. Cell death is a consequence of many factors, and more highly structured models take into account cell age, concentrations of toxic substances, pH, and other conditions. The simplest model is that which incorporates a first-order cell death term into the rate equation, so that the overall rate equation becomes

$$r_x = \mu_m \frac{SX}{S + K_s} - k_d X, \quad (5.7)$$

where  $r_x$  = rate of growth of cells, mass or number per liter per unit time;

$X$  = concentration of cells, mass or number per liter;

$k_d$  = specific death rate constant, reciprocal time units.

The Monod equation by itself does not predict any population limitation. Nor, without a death rate term, does it predict a stationary growth phase. An equation that does predict a stationary phase without inclusion of a death rate term is the logistic, or Verhulst-Pearl (Verhulst, 1839; Pearl and Reed, 1920), equation:

$$r_x = \frac{dX}{dt} = \mu_m X \left[ 1 - \frac{X}{X_t} \right], \quad (5.8)$$

where  $X_t$  = terminal cell concentration.

As discussed by Slater (1985), this simple empirical population model predicts a stationary phase at maximum population without inclusion of a death rate term. It is a satisfactory model in many cases.

## STOICHIOMETRY FOR SUBSTRATE AND PRODUCT RELATIONSHIPS

Rate equations based on cell masses or numbers usually must be translated into rate equations for depletion of substrate or production of product. This translation ordinarily is handled through so-called yield factors that represent ratios of cell mass production, product formation, substrate utilization, oxygen utilization, and the like. In the majority of cases these yield factors are handled as simple constants. In more complex structured models of nonhomogeneous cultures, they are far from constant. When cells are actively dividing, growth and division processes are their predominant activities. Even nongrowing cells actively maintain their integrities within their surroundings. The two primary categories of cell activity are growth and maintenance. Yield factors determined simply from the various metabolite ratios can differ widely, depending on which activity predominates. The usual practice is to define basic yield factors as those for the growth-associated processes and then to modify them for combinations of growth and maintenance.

Examples of yield factors are

$Y_{p/x}$  = ratio of mass of product produced per mass of cells produced;

$Y_{x/s}$  = ratio of mass of cells produced per mass of substrate converted;

$Y_{s/x}$  = reciprocal of  $Y_{x/s}$ ;

$Y_{p/s}$  = ratio of amount of product produced to amount of substrate consumed, mass/mass or mole/mole.

Different practitioners apply somewhat different yield factors, so the designer must ascertain the exact definition of the factors before using them. For utilization of substrate, the rate equation for Monod kinetics is

$$r_s = - \frac{dS}{dt} = Y_{s/x} \mu_m \frac{SX}{S + K_s} + mX, \quad (5.9)$$

where  $m$  = maintenance coefficient, time<sup>-1</sup>;

$r_s$  = volumetric substrate consumption rate, moles or mass per liter per unit time.

The first term on the right-hand side of Equation 5.9 shows the substrate utilization rate as dependent on the rate of cell multiplication, whereas the second term expresses the substrate utilization rate as dependent on the number of cells. Typically,  $Y_{x/s}$  is on the order of 0.5 (g dry cell mass)/(g substrate), whereas the comparable  $m$  could be on the order of 0.02 g substrate/[(g dry cell mass)(hour)] for mesophilic organisms. Matsché and Andrews (1973) showed that, for at least one strain of thermophilic *Bacillus* the maintenance factor could become quite high, increasing from 0.033 (g substrate)/[(g dry cell mass)(h)] at 45.5 °C to 0.145 (g substrate)/[(g dry cell mass)(h)] at 64.5 °C. Maintenance factors depend on the nature of the medium in many cases (Stouthamer and Van Verseveld, 1985). Rearranging Equation 5.9 (Humphrey, 1986) gives

$$-\frac{1}{X} \frac{dS}{dt} = \left( \frac{1}{Y_{x/s}} \right) \left( \frac{1}{X} \right) \frac{dX}{dt} + m. \quad (5.10)$$

A plot of  $-(1/X) (ds/dt)$  versus  $(1/X) (dX/dt)$  then gives a straight line with an ordinate intercept of  $m$  and a slope of  $(1/Y_{x/s})$ . Such a plot can be prepared from rates of substrate depletion and cell production at various cell concentrations. Yield factors and maintenance coefficients are both functions of temperature, with  $m$  and  $Y_{s/x}$  both usually increasing with temperature. Roels (1983a) reports that  $m$  is much more temperature dependent than  $Y_{x/s}$  and that for the average maintenance factor an Arrhenius type equation may be written as

$$m = C \exp \left[ \frac{-40,000(\text{kJ/mol})}{RT} \right], \quad (5.11)$$

where  $C$  = preexponential factor, C-mole/(mole dry mass)(h);

$R$  = 8.314 J/mol·K;

$T$  = temperature, K.

Many cells adapt to their environments through the process of induction, in which enzymes are produced at higher rates because of derepression of genes. In cases where cells can use multiple substrates, this adaptation can result in a complete switch from the preferred substrate to the alternative

choice(s) upon depletion of the primary substrate. In these special (but not unusual) cases, yield and maintenance factors may become specific functions of the medium compositions and time.

Product formation may be growth-associated or not. Gaden (1959) classified fermentation processes as three kinetic groups or types:

1. Type I processes are those in which the main product is formed as a result of primary energy metabolism. Examples are aerobic yeast cell production, alcoholic fermentation to produce ethanol, glucose oxidation to gluconic acid, and conversion of sugar to lactic acid.
2. Type II processes are those in which the main product appears indirectly from energy metabolism reactions. Examples are formation of citric acid, certain amino acids, and itaconic acid.
3. Type III processes are those in which the main product does not come from primary energy metabolism but is independently elaborated or accumulated. Examples are synthesis of antibiotics, such as penicillin or streptomycin, and accumulation of carbohydrate polymers, proteins or fats.

The formation of Type I or Type II products is by so-called dissimilation reactions with negative Gibbs free energies of reaction. The formation of complex products through Type III processes involves reactions with positive Gibbs free energies of reaction. Type I process products are growth-associated (Gaden, 1959). Type II products may not be produced until a second phase of growth. Thus they can have production rates that may or may not be associated with growth but may be more or less associated with primary substrate uptake. In Type III processes the primary growth phase usually does not result in accumulation of any of the main product. The main product commonly appears after the initial growth phase and uptake of primary substrate. Although Type III products, especially the antibiotics, are important, modeling Type III processes is difficult and specific for the process involved. Initial growth environments condition the cells for subsequent product formation, so modeling the growth phase is important even though the main product is not produced until later phases. Product formation in the simpler Type I and Type II processes can be simulated with equations containing two terms. For the simplest general case—in which product production has both growth-related and nongrowth-related components—Equation 5.12 may be applied:

$$r_p = \frac{dp}{dt} = Y_{P/X} \mu_m \frac{SX}{K_s + S} + BX, \quad (5.12)$$

where  $B$  = cell-mass related production factor, time<sup>-1</sup>;

$r_p$  = volumetric product formation rate, moles or mass per unit volume per unit time.

In many cases, working with specific rates is more convenient than working with volumetric rates. The specific substrate consumption rate,  $q_s$ , is

$$q_s = \frac{r_s}{X}. \quad (5.13)$$

The specific product formation rate,  $\nu$ , is

$$\nu = \frac{r_p}{X}. \quad (5.14)$$

In the general definitions, yield factors, maintenance coefficients, and cell-related production factors all should be considered to be variable point functions. For instance,  $Y_{x/s}$  is strictly defined at a point as

$$Y_{x/s} = -\frac{dX}{dS} = \frac{-\frac{dX}{dt}}{\frac{dS}{dt}}. \quad (5.15)$$

If any or all of these factors are variable, net time yields give different overall yield factors.

Yield factors have significance in their relationship to process efficiency. Efficiency, like beauty, is in the eye of the beholder. For example, in aerobic production of yeast, a high  $Y_{x/s}$  is desired. In anaerobic conversion of glucose to ethanol by yeast, a high  $Y_{P/s}$  is the goal. This latter goal usually coincides with minimization of  $Y_{x/s}$ . There are many definitions for specific biological process efficiencies, including, for example, those for efficiency of alcoholic fermentation discussed by Parsons (1984). In this process, maximizing ethanol yield is desirable, and there are some practical limits to conversion. This process essentially is growth-associated, so some minimal amount of glucose feed (about 2%) must go into cellular material. Some by-products always are made, and cells usually leave some glucose unconverted. The question then becomes what to use as the base for 100% conversion. Parsons (1984) used the following equation to define fermentation efficiency, FE(%):

$$FE(\%) = \frac{\text{moles ethanol formed}}{\text{moles glucose consumed}} \times \frac{100}{2}, \quad (5.16)$$

where the factor (1/2) is a consequence of the theoretical yield of two moles ethanol per mole of glucose by this metabolic pathway. The definition expressed in Equation 5.16 gives a practical value of efficiency that never reaches more than about 95% for the reasons stated. Note the relationship of the molar

factor,  $Y_{P/s}$ , to fermentation efficiency, FE(%), which can be stated more succinctly as

$$\text{FE}(\%) = 50Y_{P/s}, \quad (5.17)$$

where  $Y_{P/s}$  = ratio of moles of ethanol produced to moles of glucose consumed.

This is but one example of the use of a yield factor to determine process efficiency. Use of any of the other definitions of efficiency discussed by Parsons can be justified. Yield factors also have obvious values in material balance calculations for reactor design.

## ALTERNATIVE MATHEMATICAL MODELS

The Monod equation is the model most often used for simulation of single-cell culture growth rates. However, it does not always match actual growth rates, and, in fact for most systems to which it is applicable, it may be so for only a limited range of conditions. Thus many other models have been developed. They range from the simplest unstructured (completely lumped into one simple statement) models for unsegregated (treated as a completely uniform population) cells to the most complex structured models with terms for all known metabolic functions limiting growth and for groups of cells segregated by differing cell sizes, ages, and other characteristics.

Derivation of models starts with obvious facts, the first being data relating growth rate to cell and substrate concentration. From these data other information can be developed. Cell growth stops at zero substrate concentration, cells utilize secondary substrates, or *cryptic* growth involving endogenous metabolism of cellular material from breakdown of other cells occurs. Substrate concentration changes at high substrate concentrations may have negligible effects on cell growth. Cell death may take place under all or perhaps only under selected conditions, and secondary metabolic states may be induced by various factors. The designer should consider all these influences when developing the model for any system.

The simpler models have in common the assumption that some essential process in cell multiplication is limiting. As discussed by Kolker (1987), these models have been named L-systems, perhaps because they are based on limiting factors or because they were discussed initially by Liebig. Some of these equations were presented by Kolker (1987), Equations 5.18–5.20, and others by Bailey and Ollis (1986), Equations 5.21–5.23.

Logistic, Verhulst–Pearl:  $\frac{dX}{dt} = k\left(X - \frac{X}{g}\right)$       (5.18)

$$\text{Gompertz: } \frac{dX}{dt} = kX \ln\left(\frac{g}{X}\right) \quad (5.19)$$

$$\text{Richards: } \frac{dX}{dt} = \frac{kX}{m} \left(1 - \frac{x^m}{g^m}\right) \quad (5.20)$$

where  $g$  = maximum value of  $X$ ;

$k$  = rate constant;

$m$  = parameter between  $-1$  and  $\infty$ , but not  $0$ .

$$\text{Tessier: } \mu = \mu_{\max}(1 - e^{-s/k_s}) \quad (5.21)$$

$$\text{Moser: } \mu = \mu_{\max}(1 - K_s S^{-\lambda})^{-1} \quad (5.22)$$

$$\text{Contois: } \mu = \mu_{\max} \frac{S}{BX + S} \quad (5.23)$$

where  $B$ ,  $\lambda$ , and  $K_s$  are constants with appropriate units.

Even though some of these models are dated and empirical, they are still applicable and should be considered for use in modeling systems when simplicity is advantageous.

The simpler limiting-factor systems can be extended by application in a piecewise manner, as illustrated by Kolker (1987). He showed how a set of two equations could be used as a piecewise linear growth model, especially for cultures of *Dunaliella parva*. In Kolker's work the cultures were growing in batch mode with substrate concentration beginning at its maximum and then declining with cell growth. The first phase was called a free growth phase; the second, an exerted growth phase. The theory was that, in the free growth phase, substrate concentration had no effect on growth rate but that, in the exerted growth phase, it exerted an effect on growth rate. Consequently, simple equations successfully model several systems.

$$\text{Free growth: } \frac{dX}{dt} = kX \quad (5.24)$$

$$\text{Exerted growth: } \frac{dX}{dt} = kW S \quad (5.25)$$

where  $k$  = growth rate constant;

$W$  = yield constant;

$S$  = substrate concentration.

The value of  $S$  can be related very simply to  $X$  for the cases when  $S$  reaches zero at the maximum value of  $X$ , termed  $g$  by Kolker. In this case,

$$S = \frac{1}{Y_{X/S}}(g - X), \quad (5.26)$$

and thus Equation 5.25 becomes

$$\frac{dX}{dt} = k \frac{W}{Y_{X/S}}(g - X). \quad (5.27)$$

So the combination of Equations 5.24 and 5.27 becomes a simplified piecewise linear growth model. Equation 5.24 describes the batch growth rate from time zero to the point when the growth rate determined by it exceeds that from Equation 5.27. Then Equation 5.27 describes the growth rate from that point on. Such a linear representation of growth has obvious mathematical advantages.

## TEMPERATURE EFFECTS ON CELL PROCESSES

Temperature affects cell processes in various ways. The most obvious effects are in actually killing cells with higher temperatures and freezing them at lower temperatures. In the range between these extremes temperature affects concentrations of gaseous reactants and products, mass-transfer rates outside and inside the cells, changes of state of reactants, and, selectively, cell reproduction and other metabolic processes. The Monod equation is an extension of the Michaelis-Menten equation as applied to the limiting process for utilization of substrate for cellular reproduction. Temperature effects on rates of cell reproduction are explained by the same reasoning applied to enzymes (Chapter 3, Effect of Temperature on Enzymatic Reactions). The curves for effect of temperature on cell reproduction rate have the same general form as those for enzyme reaction rate. (See Figure 3.8.)

Microorganisms are classed according to the temperature range within which their optimum growth rate occurs. The general optimal temperature ranges applied are 55–75 °C for thermophiles, 30–45 °C for mesophiles, 25–30 °C for facultative psychrophiles, and 15–18 °C for obligate psychrophiles (Stainer, Adelberg, and Ingraham, 1976). Some thermophiles found in volcanically heated ocean streams can multiply at temperatures far above the normal range. In general thermophilic organisms have base rates higher than comparable mesophilic microorganisms, so efforts are continually made to produce or isolate thermophilic mutants. Mammalian cells and plant cells are generally mesophilic, and reproduction of viruses or other nucleic acids within such cells can be very temperature sensitive, especially for the animal cells. Bioreactor design usually is best planned for operation at somewhere near the temperature giving the maximum cell reproduction rate but not so close that it is likely to

cause cell death or adversely affect reactants or products. Adverse temperature effects may occur in large reactors in other than laboratory systems because of the great differences in mixing and heat transfer between the two systems.

Elevated temperatures are used to advantage in sterilization and pasteurization processes. Such processes are most often modeled as simple first-order decay processes:

$$\frac{dN}{dt} = -k_d N \quad (5.28)$$

and

$$k_d = A_d \exp\left(\frac{-E_d}{RT}\right), \quad (5.29)$$

where  $A_d$  is the Arrhenius preexponential factor,  $\text{s}^{-1}$  and  $E_d$  is the activation energy,  $\text{J/mol}$ .

Spores are much more resistant than vegetative cells, especially when exposed to dry heat. In fact, some are even activated to growth when exposed to temperatures sufficient to kill most vegetative cells. Consequently, wet sterilization is generally preferred to dry sterilization. The value of  $E_d$  is relatively large—on the order of 50–100 kcal/mol (Bailey and Ollis, 1986). Thus small changes in sterilization temperature can greatly affect death rate, and advantage can be taken of differences in rates of denaturation of desirable constituents and killing of undesired cells.

## EFFECTS OF OTHER ENVIRONMENTAL FACTORS

Besides temperature, pH probably has the greatest effect on cell growth kinetics. Consequently, control of pH in laboratory rate studies is essential. Departure of pH from the optimum for animal cells results in dead cells in many cases; pH values slightly above neutral are generally preferred by animal cells. Most bacteria show pH optima in the 6.5 to 7.5 range, but there always are exceptions. Some sulfur-oxidizing bacteria can exist at pH values down to about 2.0. Fungi grow over a wider range of pH values than other classes of cells, but the optima usually fall in the range of 5 to 7 for molds and 3 to 5 for yeasts.

In open cultures, such as those encountered in activated sludge, effects of pH changes on microbiotic population distribution include drastic changes in flocculation and odor when pH values are allowed to drop low enough to favor the yeasts and molds. Of course, pH optima can be strain specific, which could

be one reason for widely differing reported pH optima even for organisms as common as *S. cerevisiae* (Parsons, McDuffie, and Din, 1984). Moreover, pH can profoundly affect product distribution from pure cultures. Holt, Cairns, and Morris (1988), for example, reported that, whereas the bacterium *C. puniceum* produced butan-1-ol as the major product from glucose substrate at pH 5.5, it produced virtually no alcohols at pH 7, producing, instead, high concentrations of acetic and butyric acids.

Ionic strength, hydrostatic pressure, dissolved oxygen content, and redox potential are other important factors for various cultures. Designers often overlook the effect of dissolved salt concentration on ionic strength when formulating growth media and providing for pH control. Overly active control of pH by wide swings in acid and base addition may cause excessive salt buildup, which is deleterious to some cultures. Control swings can also cause detrimental effects on operations involving high pressures. Cells that can withstand high pressures often are injured by fast pressure changes. Obligate anaerobic cells are adversely affected by the presence of dissolved oxygen, whereas aerobic cells require a minimal concentration for multiplication. Trace minerals so essential for growth by most cells become toxic at low concentrations.

## CELL GROWTH INHIBITION

Because inhibition of cell growth can usually be traced to inhibition of specific enzymatic reactions, the models used for inhibition of enzymatic reactions (Chapter 3) also may be used for inhibition of cell growth. Cell growth may be inhibited by factors other than specific enzyme inhibitors. Specific agents may cause changes in cell permeability; stop division, transcription, or translation of DNA; or cause clumping of cells. Such cases usually require special treatment. Substrates or products also may inhibit cell growth, and products such as ethanol can even kill the cells producing them. Simple models do not suffice for simulation of these cases either. Finally, cells can inhibit growth of the cell population when it becomes too high.

Han and Levenspiel (1988) presented a summary of many of the Monod-type equations used to model systems inhibited by substrate, product, or cells. Andrews (1968) presented an equation based on a model by Haldane for substrate inhibition:

$$\mu = \mu_m \frac{S}{S + K_s + \frac{S^2}{K_i}}, \quad (5.30)$$

where  $K_i$  = inhibition constant, M.

Alternatively, Equation 5.30 can be expressed as

$$\mu = \mu_m \left( \frac{1}{1 + \frac{K_s}{S} + \frac{S}{K_i}} \right) \quad (5.31)$$

or

$$\frac{1}{\mu} = \frac{1}{\mu_m} \left( 1 + \frac{K_s}{S} + \frac{S}{K_i} \right), \quad (5.32)$$

the latter known as the Lineweaver–Burk form. Typically, a plot of  $\mu$  versus  $S$  gives a maximum as discussed by Han and Levenspiel (1988). The maximum  $\mu$  occurs at  $S = \sqrt{K_i K_s}$ . Substrate inhibition is less likely to be important in continuous stirred-tank reactors (CSTR) than in batch or plug-flow reactors because of the reduced substrate concentration from backmixing.

The basic models for inhibition clearly show some of the difficulties encountered in modeling reactors for cell culture. As discussed in Chapter 3, the standards derived from a Michaelis–Menten analysis of steady-state dissociation models are identical in form to the analogous equations for enzyme inhibition. The more common forms are

- *For uninhibited substrate utilization*

$$\mu = \mu_m \frac{S}{K_s + S} \quad (5.6)$$

- *For competitive inhibition*

$$\mu = \mu_m \frac{S}{S + K_s \left( 1 + \frac{I}{K_{ic}} \right)} \quad (5.33)$$

- *For uncompetitive inhibition*

$$\mu = \mu_m \frac{S}{K_s + S \left( 1 + \frac{I}{K_{iu}} \right)} \quad (5.34)$$

- *For mixed inhibition*

$$\mu = \mu_m \frac{S}{K_s \left( 1 + \frac{I}{K_{ic}} \right) + S \left( 1 + \frac{I}{K_{iu}} \right)} \quad (5.35)$$

- For pure noncompetitive inhibition

$$\mu = \mu_m \frac{S}{(K_s + S) \left( 1 + \frac{I}{K_i} \right)} \quad (5.36)$$

The models for competitive and pure noncompetitive inhibition, Equations 5.33 and 5.36, respectively, are forms of the Monod equation modified by multipliers. For competitive inhibition, the model in Equation 5.33 is effectively the Monod equation with a modified value of  $K_s$ , or

$$K_{s\text{eff}} = K_s \left( 1 + \frac{I}{K_{ic}} \right), \quad (5.37)$$

where  $K_{s\text{eff}}$  = effective modified value of  $K_s$ .

For pure noncompetitive inhibition,  $\mu_m$  is modified by a multiplier, or

$$\mu_{m\text{eff}} = \mu_m \left( \frac{1}{1 + \frac{I}{K_i}} \right), \quad (5.38)$$

where  $\mu_{m\text{eff}}$  = effective modified form of  $\mu_m$ .

These Monod-type equations can be analyzed by use of the Lineweaver-Burk reciprocal plots in the same manner as the corresponding Michaelis-Menten forms for enzyme inhibition. The Monod-type equations are convenient, they have an accepted basis in theory, and they have been used extensively. In many cases, however, they should not be used for the entire range of inhibitor concentrations, especially for extended cultures of cells suffering product inhibition. Cultures producing high concentrations of toxic products create special difficulties. One example is the production of ethanol from fermentation of glucose by *S. cerevisiae* (Parsons, 1984). This process can be modeled quite well with Monod noncompetitive-type kinetics up to the higher ethanol concentrations, but there it begins to fail. The Monod model predicts continuing ethanol production and cell multiplication above ethanol concentrations that completely inhibit both processes in experimental runs. This situation is not remedied by the linear inhibition model,

$$\mu_m = \mu_o - K_i P, \quad (5.39)$$

where  $K_i$  = inhibition constant, L/(mol·s);

$\mu_o$  = uninhibited value of  $\mu_m$ , s<sup>-1</sup>.

Nor is the situation handled by one of the exponential forms of equations, such as

$$\mu_m = \mu_o e^{-K_i P}, \quad (5.40)$$

where  $K_i$  = inhibition constant, L/mol.

Two possible remedies for this dilemma are suggested. One is to create a structured model reflecting that (a) the intracellular ethanol content is higher than the extracellular concentration (Nagodawithana and Steinkraus, 1976; Navarro and Durand, 1978; Thomas and Rose, 1979; Panchal and Stewart, 1980; Novak *et al.*, 1981); (b) there are differences in inhibition effects of intracellular and extracellular ethanol (Hoppe and Hansford, 1982); (c) growth rates and ethanol production rates are affected differently by ethanol inhibition; and (d) higher concentrations of ethanol have a *killing effect* on the yeast (Brown *et al.*, 1981; Righelato *et al.*, 1981). Development of accurate models taking all these factors into account must await detailed strain-specific kinetic data. In the meantime, as the second remedy, equations of an empirical nature such as those presented by Han and Levenspiel (1988) provide satisfactory models. They proposed the following general type of equation for substrate, product, and cell inhibition for values of  $C_i$  up to  $C_i^*$ :

$$\mu = \mu_m \left(1 - \frac{C_i}{C_i^*}\right)^n \frac{S}{S + K_s \left(1 - \frac{C_i}{C_i^*}\right)^m}, \quad (5.41)$$

where  $C_i$  = inhibitor concentration, M;

$C_i^*$  = limiting inhibitor concentration, M;

$n, m$  = constants.

Use of the ratio,  $C_i/C_i^*$ , provides for the cases in which cell production stops completely at  $C_i \geq C_i^*$ . The equations, of course, are not good for  $C_i > C_i^*$ . The choice of values of  $n$  and  $m$  accounts for

1. pure noncompetitive inhibition when  $n > 0$  and  $m = 0$ ;
2. competitive inhibition when  $m < 0$  and  $n = 0$ ; and
3. mixed inhibition when  $m \neq 0$  and  $n \neq 0$ .

The Han–Levenspiel equation becomes Equation 5.42 for substrate inhibition and Equation 5.43 for cell inhibition:

$$\mu = \mu_m \left(1 - \frac{S}{S^*}\right)^n \frac{S}{S + K_s \left(1 - \frac{S}{S^*}\right)^m}; \quad (5.42)$$

$$\mu = \mu_m \left(1 - \frac{X}{X^*}\right)^n \frac{S}{S + K_s \left(1 - \frac{X}{X^*}\right)^m}; \quad (5.43)$$

where  $S^*$  = limiting substrate concentration, M;  
 $X^*$  = limiting cell concentration, g/L or cells/L.

Han and Levenspiel (1988) showed how constants for their equations can be determined for the various types of inhibition. First, cell growth rates at various combinations of  $S$  and  $C_i$  have to be determined. These rates are used in Lineweaver–Burk-type plots to determine “observed” values of  $\mu$  and  $K_s$ . The Lineweaver–Burk plot is commonly used to determine constants for the Michaelis–Menten equations for enzymatic reactions, as discussed in Chapter 3. The Michaelis–Menten equation (Equation 3.2) can be inverted to give the Lineweaver–Burk equation:

$$\frac{1}{v} = \frac{K_{ma}}{V} \cdot \frac{1}{S} + \frac{1}{V}. \quad (3.38)$$

Application of Equation 3.38 is commonly a linear plot of  $1/v$  versus  $1/S$  to determine  $K_{mA}$  and  $V$  from intercepts, as  $1/v = 1/V$  at  $1/S = 0$ , and  $1/K_{mA} = -1/S$  at  $1/v = 0$ . The form of the Monod equation is identical to that of the Michaelis–Menten equation, so plots of  $1/\mu$  versus  $1/S$  can be used to obtain  $\mu_m$  and  $K_s$  from linear plots of

$$\frac{1}{\mu} = \frac{K_s}{\mu_m} \left(\frac{1}{S}\right) + \frac{1}{\mu_m}. \quad (5.44)$$

In the Han–Levenspiel equations,  $C_i/C_i^*$  is constant if  $C_i$  is maintained constant. Then, Monod equations can be set up with “observed” values of  $\mu_m$  and  $K_s$ . Upon inversion, Equation 5.41 becomes

$$\frac{1}{\mu} = \frac{1}{\mu_m \left(1 - \frac{C_i}{C_i^*}\right)^n} \left[1 + K_s \left(1 - \frac{C_i}{C_i^*}\right)^m \left(\frac{1}{S}\right)\right]. \quad (5.45)$$

So in a plot of  $1/\mu$  versus  $1/S$  for constant  $C_i$ ,

$$\left(\frac{1}{\mu_m}\right)_{\text{obs}} = \frac{1}{\mu_m \left(1 - \frac{C_i}{C_i^*}\right)^n} \quad (5.46)$$

and

$$(K_s)_{\text{obs}} = K_s \left(1 - \frac{C_i}{C_i^*}\right)^m. \quad (5.47)$$

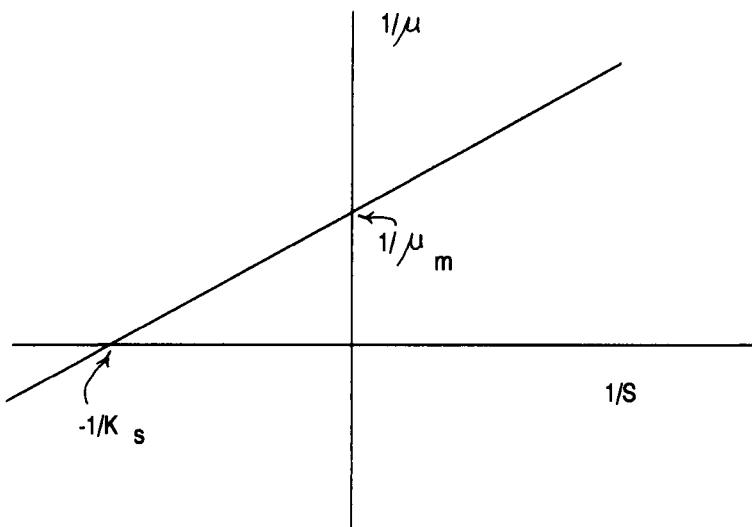
For each  $C_i$ , “observed” values of  $(1/\mu_m)$  and  $K_s$  may be determined from plots such as those in Figure 5.2. The logarithmic forms of Equations 5.46 and 5.47 relating “observed” Monod equivalent values of  $K_s$  and  $\mu_m$  to Han–Levenspiel values are

$$\ln(\mu_m)_{\text{obs}} = \ln \mu_m + n \ln \left(1 - \frac{C_i}{C_i^*}\right) \quad (5.48)$$

and

$$\ln(K_s)_{\text{obs}} = \ln K_s + m \ln \left(1 - \frac{C_i}{C_i^*}\right). \quad (5.49)$$

If  $C_i^*$  is known from experiment (as the minimum concentration of inhibitor completely stopping growth),  $n$  and  $\ln \mu_m$  can be obtained from a plot of  $\ln(\mu_m)_{\text{obs}}$  versus  $\ln(1 - C_i/C_i^*)$  as slope and ordinate intercept, respectively, following Equation 5.48. Next,  $m$  and  $\ln K_s$  may be obtained from a plot of  $\ln(K_s)_{\text{obs}}$  versus  $\ln(1 - C_i/C_i^*)$  as slope and  $y$  intercept, respectively, following



**FIGURE 5.2** Lineweaver–Burk plot for determining observed values of  $K_s$  and  $\mu_m$  for Monod kinetics.

Equation 5.41. Han and Levenspiel (1988) show how  $C_i^*$  can be determined by trial and error if it is not known directly from experiment. Incorrect values of  $C_i^*$  give curved rather than straight lines of the plots of  $\ln(K_s)_{\text{obs}}$  versus  $\ln(1 - C_i/C_i^*)$ , so that the test for proper value of  $C_i^*$  is that it gives a straight-line plot.

For the Han–Levenspiel substrate inhibition model (Equation 5.42), constants must be evaluated somewhat differently, because substrate and inhibitor are the same substance. As  $S$  approaches  $S^*$ , Equation 5.42 is approximated by Equation 5.50:

$$\mu \cong \mu_m \left(1 - \frac{S}{S^*}\right)^n. \quad (5.50)$$

Values of  $n$  and  $\mu_m$  can be obtained by application of plots of  $\ln \mu$  versus  $\ln(1 - S/S^*)$  for high values of  $S$  if  $S^*$  is known. Otherwise,  $S^*$  may be determined by finding the value of  $S^*$  that gives a straight-line plot and then finding  $n$  and  $\ln(\mu_m)$  as slope and y intercept, respectively. Next,  $m$  and  $K_s$  can be determined at low substrate values in the same manner as for the general inhibition model.

There are many other good inhibition models. The Monod-type equations are generally satisfactory in systems in which high concentrations of inhibitors are not encountered. Constants for the various equations usually have been evaluated with data from batch growth systems. In these systems concentrations of cells, metabolites, and inhibitors are not constant and thus inaccuracies arise. Data from continuously stirred tank biological reactors are better if available, because conditions may be adjusted in such reactors.

## MASS-TRANSPORT AND PHYSICAL RESTRAINT LIMITATIONS

The mathematical models for cell growth discussed to this point have no physical restraints (except contact inhibition) and no mass-transport limitations. In many cases for single-cell cultures this assumption is satisfactory. Many mass-transport limitations can be eliminated or minimized by proper reactor design. Such is not the case, however, in growth systems involving thick mats of cells, flocs, large pellets, or immobilized cells. In addition to mass-transport problems, there can also be physical restraints to cell reproduction. For example, only so many cells can occupy a fixed pore volume in an immobilizing structure. This condition naturally is an advantage if minimizing cell multiplication is the goal.

Before the advent of extensive use of immobilized enzyme and cell systems in bioreactors, consideration of mass transport centered on oxygen transport in aerobic systems. Although oxygen is only slightly soluble in aqueous systems, cell growth is generally not limited until oxygen concentration in the medium is reduced considerably below the concentration in equilibrium with air at

atmospheric pressure. At an oxygen content termed the *critical oxygen tension*, or critical oxygen value, it does become limiting, and effects can be modeled with a Monod-type equation for oxygen if other growth requirements are met. The critical oxygen content ranges from  $3 \times 10^{-6}$  M to  $5 \times 10^{-5}$  M (Bailey and Ollis, 1986), or a range from about 1% to 25% of air saturation values (Figure 2.1). As outlined in Chapter 4, oxygen flux is usually limited by liquid film resistance. If the critical oxygen concentration is assumed to be the minimum allowable oxygen concentration in the reactor, Equation 4.9 becomes

$$N_{O_2} = k_L(C_{O_2}^* - C_{CR_{O_2}}) \quad (5.51)$$

where  $C_{CR_{O_2}}$  = critical oxygen concentration.

The total rate of oxygen transport per unit volume of medium then is

$$N_{O_2}a = k_La(C_{O_2}^* - C_{CR_{O_2}}). \quad (5.52)$$

Two primary factors determine the relative importance of oxygen transport—namely, oxygen rate requirement and transport driving force. The total oxygen rate requirement is a function of cell concentration, metabolic rate, and degree of substrate reduction. The relative driving force from Equations 5.51 and 5.52 is given by  $(C_{O_2}^* - C_{CR_{O_2}})$ . The most demanding situation is a high oxygen rate requirement with a low driving force. Some *Penicillium* molds growing on simple carbohydrates qualify on both counts. They have high critical oxygen concentrations and high total oxygen rate requirements. Paraffin hydrocarbons have the highest degrees of reduction, and thus microorganisms in vigorous aerobic growth states on hydrocarbons have demanding mass transport design requirements. Growth on methane compounds the problem, because methane, like oxygen, is a sparingly soluble gas. Interesting models can be developed for growth of cell colonies or mycelia limited to certain dimensions. Growth in a pore of a support may be limited to one dimension, growth on a plane or spherical surface essentially to two dimensions, and so on.

Many molds grow in essentially spherical *pellets*, but pellet growth does not generally follow Monod kinetics. One of the simplest explanations of this departure is to assume that growth is limited physically to the outer surface. For this case, let

$V_p$  = volume of pellet;

$r_p$  = radius of pellet;

$S_p$  = surface area of pellet.

Then

$$V_p = \frac{4}{3}\pi r_p^3; \quad (5.53)$$

$$S_p = 4\pi r_p^2. \quad (5.54)$$

The rate of growth is then proportional to the outer surface area, so

$$\frac{dV_p}{dt} = kS_p, \quad (5.55)$$

where  $k$  = arbitrary constant for linear growth.

From Equations 5.54 and 5.55,

$$\frac{dV_p}{dt} = k(4\pi r_p^2) \quad (5.56)$$

and

$$\frac{dV_p}{dt} = (36\pi)^{1/3}kV_p^{2/3}. \quad (5.57)$$

The integrated form of Equation 5.57 then is

$$V_p^{1/3} = \frac{(36\pi)^{1/3}}{3}kt + C \quad (5.58)$$

or

$$V_p^{1/3} = \frac{(36\pi)^{1/3}}{3}kt + V_{p0}^{1/3}, \quad (5.59)$$

where  $V_{p0}$  = pellet volume at time zero.

As discussed by Bailey and Ollis (1986), if the pellets are small at time zero, the simplified form of Equation 5.59 shows  $V_p$  to be a cubic function of time:

$$V_p \approx 4\pi(kt)^3. \quad (5.60)$$

An alternative explanation of the limitation of pellet growth was offered by Pirt (1967): Pellet growth is limited by mass transfer of an essential metabolite, most likely oxygen. As originally proposed by Pirt (1967) and discussed by Righelato (1975) and Moo-Young and Blanch (1983), analysis of growing cultures leads to the conclusion that a peripheral growth zone about 0.077 mm

thick exists in mycelial pellets. This conclusion develops from an analysis starting with the initial assumption that growth is exponential in the active zone so that

$$\frac{dX}{dt} = \mu X_p, \quad (5.61)$$

where  $X$  = mass of pellet;

$X_p$  = mass of active peripheral zone.

Moreover,

$$\frac{dr_p}{dt} = \mu w,$$

where  $r_p$  = radius of pellet;

$w$  = thickness of peripheral growth zone;

Finally,

$$r_p = \mu wt + r_0, \quad (5.62)$$

where  $r_0$  = pellet radius at time zero.

For pellets of mass density,  $d$ ,

$$X = \frac{4}{3}\pi r^3 d, \quad (5.63)$$

where  $X$  = pellet mass.

Combining Equations 5.62 and 5.63 yields

$$X^{1/3} = \left(\frac{4\pi d}{3}\right)^{1/3} \mu wt + X_0^{1/3}, \quad (5.64)$$

where  $X_0$  = pellet mass at time zero.

The net outcome is still a cubic dependence of pellet mass on time.

The limitations of geometry, intrinsic cell growth capacity, substrate and nutrient concentrations, and mass transfer are incorporated in the net bioreactor design model. Fortunately, in many cases only one slower process limits cell growth and simpler models can be used. None of the possible limits, however, should be overlooked, especially when equipment is scaled up.

## MIXED CULTURES

Growth of cells in nature generally does not occur in pure cultures. The multiplication of microorganisms in mixed systems deserves some attention because of the significance in natural environments and in various synthetic environments. Activated sludges used in waste treatment are diverse mixtures of organisms. Their usefulness depends on the range of activities offered by the different organisms present. Mixed cultures result from contamination of one with another, and the nature of the interaction determines whether such mixing is a disaster or is controllable.

In general, the cultures used in anaerobic and aerobic waste treatment can be modeled by the use of conventional Monod kinetics. Monitoring and control of the cultures to maintain proper environmental quality for a healthy mixture are important. Cultures of pairs of microbe types offer intriguing mathematical challenges, because cultures are generally cyclic or unstable. Use of such systems under a controlled environment to accomplish specific conversions will certainly become more common as knowledge of control systems advances.

Fredrickson (1983) discussed various classes of binary microbial interactions. Briefly, for interaction of populations A and B, the types are:

- *Competition* A and B compete for resources (common substrate, oxygen, etc.).
- *Antagonism* A and B express negative effects on each other by producing toxins or inhibitors.
- *Amensalism* Only one of the pair causes negative effects on the other by producing toxins or inhibitors.
- *Eccrinolysis* One of the pair causes solubilization of biomass of the other by producing lytic agents.
- *Commensalism* One of the pair enhances growth of the other by producing growth-stimulating agent or removing growth inhibitor.
- *Protocooperation* Protocooperation is the same as commensalism, except that both enhance each other's growth.
- *Mutualism* Mutualism is the same as protocooperation, except that presence of both populations is required for either to grow.
- *Feeding, including predation and suspension-feeding* One population feeds on the other; in predation the predator actively pursues its prey.
- *Symbiosis* Specific physical contact and interaction are required for one or both of the pair.
- *Parasitism* The parasite population penetrates cells of a host to convert host cell material to its own.
- *Crowding* Populations compete physically for space to grow.

Parasitism includes the relationship between viruses or bacteriophages and their hosts. Plasmids might also be classed as parasites, because they function much like the viruses (with much outside help required). The parasitic rela-

tionship is important for two reasons: (a) Investigators are finding more and more uses for viruses, phages, or plasmids as vectors for production of bioengineered proteins; and (b) more conventionally, virus and bacteriophage contamination can wreak havoc on cultures of all types of cells. In some cases production of the viruses or phages is desired, but, when it is not, eliminating them from cultures is difficult. Phages attack microbial cultures, and even the mycoplasmas are not excepted. Microbial cultures develop resistance to attack through mutation and selective adaptation, but the attackers possess the ability to adapt as well. Usually the only way to eliminate undesired phage contamination is to start with a new cell culture.

The bacteriophages, viruses, and plasmids reproduce by utilizing cellular synthetic apparatus. The relationship of their reproduction to cell cycles differs from one culture to another. The important difference between kinetics of cell multiplication and duplication of these subcellular invaders is that many particles can be produced from one invaded cell. Thus the production model must account for this production of a *burst* of particles from one seed rather than the simple fission model used for cell duplication.

## AUTOTROPHIC GROWTH

Autotrophic growth models are similar to those used for heterotrophic growth, but specific types of autotrophic growth require different concepts. At times, classification uncertainties cloud issues, but these uncertainties should not cause difficulty in developing growth models. Possibly the most significant autotrophs from the bioengineering standpoint are the photoautotrophs, or photosynthetic organisms. For these and for most of the chemoautotrophs, the energy source and the carbon source are different. For photosynthesis with carbon dioxide (or bicarbonate ion) as the carbon source, the distinction between the carbon source and the energy source is clear. The rate of supply of either energy or carbon may be growth limiting for autotrophs in general. When growth is carbon limited, general growth models, such as the Monod-type equations, are used to obtain reaction rate limits. Similarly, when growth is limited by the presence of any other dissolved nutrient, the same types of kinetic models may be used. In either case, of course, mass-transfer limits must also be considered. Photosynthesis in an energy-limited environment is a special case and requires appropriate treatment.

Photosynthesis depends on an adequate supply of light of the appropriate wavelength to meet all the energy requirements of the organism (during the light phase). The overall efficiency of conversion of sunlight to energy stored in chemical bonds is low, both because of the specific wavelength requirements of photosynthetic organisms and because of the loss of energy from light absorption and conversion to heat. Some light striking the medium surface is absorbed by the medium, by cellular material, and by other suspended solids and some is reflected. Light intensity decreases logarithmically with distance

through the medium. Exact modeling of the organism–light interaction becomes a complex function of mixing in larger growth chambers. Practical depth is limited by light absorption. With very good mixing in smaller systems, the rate of growth may be correlated more easily with light intensity. Heifetz and Quinlan (1988) demonstrated how growth of the blue–green alga *Spirulina platensis* could be simulated by a Monod-type equation taking into account the organisms maintenance requirements. The form of equation presented by Heifetz and Quinlan is

$$\mu = \mu_m \frac{I - I_t}{I + K}, \quad (5.65)$$

where  $\mu$  = specific growth rate, time<sup>-1</sup>;

$\mu_m$  = maximum specific growth rate, time<sup>-1</sup>;

$I$  = light intensity,  $\mu\text{E PAR/m}^2\cdot\text{s}$ ;

$I_t$  = threshold light intensity, or compensation light intensity,  $\mu\text{E PAR/m}^2\cdot\text{s}$ ;

$K$  = Monod-type constant,  $\mu\text{E PAR/m}^2\cdot\text{s}$ .

The threshold, or compensation, light intensity,  $I_t$ , is the light intensity at which the growth rate switches from negative to positive. It is a maintenance energy intensity required for steady-state maintenance at zero growth rate and is determined as the  $x$  intercept of a plot of  $\mu$  versus  $I$ . The quality of the light is significant, and sufficient mixing, light exposure, and an adequate supply of CO<sub>2</sub> and minerals at optimal pH must be ensured before a model such as that expressed in Equation 5.65 can be used for scaling up laboratory reactors to commercial size. Otherwise, the designer may need to compensate for nutrient gradients and light intensity gradients in the system.

## RATE LIMIT DETERMINATIONS

In each culture system, the growth rate limit is a function of one or more variables. The least complex model expresses this limit as a relatively simple function of concentration of some nutrient or energy source. Actually, the nature of the limiting factor may vary during the course of a batch culture growth or changing continuous growth. For instance, there may be an intermediate product limit (as with extracellular hydrolytic enzymes) followed by an oxygen limit, a soluble phosphate limit, a substrate limit. There is no sudden jump from one limit to the other, and in each transition two or more limiting factors must be considered. The usual approach is to use Monod-type factors to correct for multiple kinetic limitations, which can be modified further for mass-transfer limitations. The mass-transfer limits effectively decrease the concentrations of nutrients available at cell surfaces or sites of reaction. Substrate,

light, mineral, vitamin, oxygen, or CO<sub>2</sub> limitations may or may not be compounded. When they are, models using Monod-type multipliers, as in the following equation, may be tried as a first approach:

$$\mu = \mu_m \frac{S_1}{K_1 + S_1} \cdot \frac{S_2}{K_2 + S_2} \cdots, \quad (5.66)$$

where  $S_1$  = concentration term for first limiting factor;

$S_2$  = concentration term for second limiting factor;

$K_1, K_2$  = constants with units of  $S_1$  and  $S_2$ , respectively.

Providing excess growth substances in the laboratory is not uneconomical. In commercial applications, however, nothing, including air, is free. Thus proper optimization involves interactive selection of all environmental variables, including mineral concentrations, oxygen supply, substrate and vitamin concentrations, and the like. As a result, more complex growth models are needed, especially when mass transfer becomes a significant limit.

# Chapter 6

---

## Enzyme Reactors

### OVERVIEW

Enzymes are the wonders of biological reaction systems. They can catalyze specific reactions at low relative temperatures. They also have greater activities than nonbiological catalysts that might just be able to catalyze the same reactions. However, the enzymes do have some drawbacks. As discussed in Chapter 3, they are usually quite labile. They are easily denatured by heat, chemical agents, or mechanical shear and may be hydrolyzed by other enzymes. They are not easily separated from products, and denatured enzymes are not easily separated from active enzymes. Many challenges thus exist in developing reactor designs to capitalize on all the advantages while eliminating or at least minimizing the disadvantages of enzymes. Probably the most extensive efforts in this direction relate to development of immobilized enzyme reaction systems. The obvious advantage of keeping the immobilized enzymes within the reaction system is not gained without costs. Even though many immobilized enzymes are more stable than the free species, others are less stable. Mass transport becomes a major consideration, whereas it usually can be neglected in the free enzyme systems.

In many cases the term *enzyme* is used for dead cells containing desirable enzymes or enzyme systems. Use of such cells has some advantages because of the content of their complex enzyme systems and cofactors. Also, they are much less expensive than purified enzymes and are more easily separated from reaction–product mixtures. Kinetic models for enzymes in dead or nonmultiplying cells are essentially the same as those for the purified enzymes.

### NONIMMOBILIZED ENZYME SYSTEMS

The nonimmobilized enzyme systems are simulated by the general kinetic models discussed in Chapter 3, and mass transfer is not usually a problem. The basic problem with nonimmobilized enzyme systems lies in the difficulty of separating the enzymes from the reactor–product mixture. In some cases the enzymes are used on a once-through basis, whether in batch or flow reactors. More expensive enzymes may be recovered by ultrafiltration, ultra-

centrifugation, dialysis, or other methods. One of the more novel reactor designs now makes use of two-phase polymer solutions in which the enzyme and reactant are selectively held in one phase while the product accumulates in the second phase. In other systems the enzymes are retained in semipermeable tubes through which the reactants are free to diffuse. In these reactors the primary design problems involve mass transport through membranes, as discussed in Chapter 4.

Standard Michaelis-Menten kinetics can be used for most free enzyme systems—but with care. In systems with high enzyme concentrations for fast reactions, the basic steady-state assumptions may not be valid. Advances in computer hardware and in software for integration of stiff differential equations, such as those encountered in dynamic-state enzyme kinetics, make simulating such fast reaction systems possible. Computer accessibility simplifies inclusion of kinetic terms for time decay of enzyme activity, reverse reactions, stepwise reactions, and the like. Thus oversimplification of kinetics can no longer be justified. However, care should be taken in the analysis of all data used in design. The Michaelis-Menten kinetic constants available are usually obtained in ideal laboratory systems for initial reaction rates and with fresh enzyme preparations. Their application in design without thorough analysis can lead to surprising results.

## IMMOBILIZED ENZYME SYSTEMS

Enzymes and cells may be immobilized in various ways, but the nature of the immobilization affects system kinetics. In many cases immobilization leads to mass-transfer limitations. If the enzymes are adsorbed or cross-linked around, or covalently bonded to, a porous support, the limit may be diffusion of substrate to the outer support surface or to inner support pore surfaces. Because of entrapment, including microencapsulation, diffusion through the entrapping structure usually becomes a limit.

Even though mass-transfer limitations are significant, especially for entrapped enzymes or enzymes in porous structures, mathematical analysis of combined intraparticle mass transfer with enzymatic reaction is largely empirical. Some qualitative analysis can be helpful in analysis of reactor performance. The usual choice, however, is just to prepare the entrapped enzyme in the smallest size particles or the thinnest membrane compatible with the flow and other physical dynamics of the reactor. Another alternative is to concentrate the enzyme in the outer layers of carrier particles in preference to the interior, which may never be penetrated by reactant. Determination of the proper dimensions (particle diameter for spheres or film thickness for coated tubes for example) normally becomes a matter of trial and error in the laboratory. As more information becomes available on diffusivities of biological reactants and products in various immobilization media, predictive models can be used with more confidence than at present. Detailed mass-transport models are discussed here with this potential in mind.

Mass transfer to and from enzyme in a slab or in particles occurs in three steps:

1. transfer of reactant through liquid film resistance at the surface;
2. transfer of reactant into pores or substance of entrapping medium with concurrent reaction and counterdiffusion of product; and
3. transfer of product through liquid film resistance at the surface.

Mass transport in the bulk fluid is accomplished by sufficient mixing. In tubular reactors with streamline flow or appreciable axial dispersion and in stirred-tank reactors with dead space or bypassing, further concentration gradients exist and must be accounted for with appropriate models. As discussed by Bird, Stewart, and Lightfoot (1960), Atkinson (1979), and Levenspiel (1989), the net results of treatment of intraparticle (or slab) diffusion with reaction for different geometric forms are close enough to each other that the kinetic correction factors developed for one can be used for the other with an error of less than 10%. This margin is safe for such qualitative calculations. For the various geometric shapes encountered, a characteristic length,  $L$ , is used:

$$L = \frac{V_p}{S_p}, \quad (6.1)$$

where  $V_p$  = volume of particle or slab;

$S_p$  = outer surface of particle or slab (usually with ends excluded).

Examples of characteristic lengths are

Sphere	$L = R/3$	$R$ = outer radius
Cylinder	$L = R/2$	Ends excluded
	$L = R/[2(1 + R)]$	Ends included
Slab	$L = \text{thickness}/2$	Two faces exposed, ends excluded
Slab	$L = \text{thickness}$	One face exposed, ends excluded

In the following discussion, a spherical particle is used as the object, but the other forms are also implied. In terms of an equivalent (or nearly so) spherical particle with radius  $R$ , the equivalent radius is

Slab	$R = 1.5(\text{thickness})$	Two exposed faces, ends excluded
Slab	$R = \text{thickness}$	One exposed face, ends excluded
Cylinder	$R = 1.5(\text{radius})$	Ends excluded
	$R = \frac{3(\text{radius})}{2 \left[ 1 + \frac{\text{radius}}{\text{length}} \right]}$	Ends included

Reactant A is supplied in bulk fluid at concentration  $C_A$ . Transport from the bulk flow to the particle's surface is through a so-called film resistance. Flux of A is described by the general equation

$$N_A = k_L(C_{AB} - C_{ASL}), \quad (6.2)$$

where  $N_A$  = flux of component A, mol/(m<sup>2</sup>·s);

$C_{AB}$  = concentration of A in bulk fluid, mol/m<sup>3</sup>;

$C_{ASL}$  = concentration of A at liquid interface with particle, mol/m<sup>3</sup>;

$k_L$  = liquid phase mass transfer coefficient, m/s.

If transport to the particle surface is much faster than transport away from the surface into the particle,  $C_{ASL}$  essentially is equal to  $C_{AB}$ . The concentration of component A within the particle at the surface is not necessarily equal to  $C_{ASL}$ , although it is generally assumed to be so unless some equilibrium determinations can be made. More properly it is the concentration in thermodynamic equilibrium with the outer surface concentration.

When transport to the particle surface is slower than transport away from the surface into the particle,  $C_{ASL}$  is less than  $C_{AB}$ . In order to evaluate the importance of the film resistance to overall transport, the designer should look at the steady-state condition for a material balance on reactant A. For a steady state to exist, the concentration of A must be constant at any point. That is, for a volume element,

$$(\Delta V_s) \frac{dC_A}{dt} = 0 = \nu_A v''' (\Delta V_s) + \text{net rate of transport of A into element}, \quad (6.3)$$

where  $C_A$  = point concentration of A;

$\nu_A$  = stoichiometric coefficient for A, negative for reactant;

$v'''$  = reaction rate, mol/m<sup>3</sup>·s;

$V_s$  = volume of solid.

If transport is viewed in a single direction in rectangular coordinates (or radial or axial direction for cylindrical coordinates or radial direction for spherical coordinates), Equation 6.3 becomes

$$\nu_A v''' (\Delta V_s) = \Delta (SN_A), \quad (6.4)$$

where  $S$  = surface area of element normal to diffusion of A;

$N_A$  = flux of A, mol/(m<sup>2</sup>·s).

Then

$$\nu_A v'''(\Delta V_s) = -\Delta \left( S D_e \frac{\partial C_A}{\partial l} \right), \quad (6.5)$$

where  $D_e$  = effective diffusivity of A in medium or pores,  $\text{m}^2/\text{s}$ ;  
 $l$  = length in direction toward center of particle, m.

For spherical particles, if  $l$  is depth into the sphere from the surface and  $r$  = radius,  $dl = -dr$ . Then for irreversible first-order reaction,

$$\nu_A k''' C_A \cdot 4\pi r^2 dl = -d \left( 4\pi r^2 D_e \frac{\partial C_A}{\partial l} \right)$$

and

$$-\nu_A k''' C_A r^2 = 2r D_e \frac{\partial C_A}{\partial r} + r^2 D_e \frac{\partial^2 C_A}{\partial r^2}$$

or

$$\nu_A k''' C_A = -\frac{2}{r} D_e \frac{\partial C_A}{\partial r} - D_e \frac{\partial^2 C_A}{\partial r^2}, \quad (6.6)$$

where  $k'''$  = first-order reaction rate constant giving rate in moles/  
 $(\text{m}^3 \text{ solid} \cdot \text{s})$ .

Following the presentation by Bird *et al.* (1960), the solution to Equation 6.6 is

$$\frac{C_A}{C_{As}} = \left( \frac{R}{r} \right) \frac{\sinh \sqrt{-\nu_A k'''/(D_e r)}}{\sinh \sqrt{-\nu_A k'''/(D_e R)}}, \quad (6.7)$$

where  $C_A$  = concentration of A at radius  $r$ ;

$C_{As}$  = concentration of A at radius  $R$  (solid side of surface).

Then the molar flow of A out of the particle in the radial direction is

$$N_{Ar} S_R = -4\pi R^2 D_e \frac{\partial C_A}{\partial r} \Big|_{r=R} \quad (6.8)$$

where  $N_{Ar}$  = flux of A in radial direction at  $r = R$ ;

$S_R$  = outer surface area of particle.

Solving for  $N_{AR}S$  using Equations 6.8 and 6.7 yields

$$N_{AR}S_R = 4\pi r D_e C_{AS} \left( 1 - \sqrt{\frac{k'''(-\nu_A)}{D_e}} R \coth \sqrt{\frac{-\nu_A k'''}{D_e}} R \right). \quad (6.9)$$

The molar reaction rate for A with no intraparticle diffusion resistance in one particle is

$$\nu_A V_s v''' = \frac{4}{3} \pi R^3 k''' \nu_A C_{AS}. \quad (6.10)$$

Division of the actual molar flow of Equation 6.9 into the particle by the theoretical maximum flow from Equation 6.10 gives an effectiveness factor, generally designated  $\eta_A$ , for reactant A:

$$\eta_A = \frac{N_{AR}S_R}{\nu_A V_s v'''} = \frac{3}{-\nu_A k''' R^2} \left( \sqrt{\frac{-\nu_A k'''}{D_e}} R \coth \sqrt{\frac{-\nu_A k'''}{D_e}} R - 1 \right). \quad (6.11)$$

Use of the definition for the so-called Thiele modulus,  $\phi$ , simplifies Equation 6.11 as follows for reactant A:

$$\phi = \sqrt{\frac{-\nu_A k'''}{D_e}} \left( \frac{R}{3} \right). \quad (6.12)$$

Recall that an equivalent value of  $R$  can be used without appreciable loss of accuracy even for films that coat surfaces. Equation 6.11 is simplified as follows by use of the Thiele modulus from Equation 6.12:

$$\eta_A = \frac{3}{\phi^2} (3\phi \coth \phi - 1). \quad (6.13)$$

The preceding analysis is built on two assumptions: (1) The reaction at the enzyme itself is first order, and (2) the spherical particle assumption is valid. Both are acceptable approximations. Recall that Michaelis–Menten kinetics vary from one extreme of zero order to the other extreme of first order for first-order association of substrate with enzyme. Most transport-limited reactions probably go from zero order to first order from the exterior of a particle to its interior. The correct solution is a numerical solution using the Michaelis–Menten kinetic expression with a finite-element calculation. However, as suggested by Bailey and Ollis (1986), Bird *et al.* (1960), and Levenspiel (1989), the efficiency calculation is relatively insensitive both to reaction order and

particle geometry. The first-order assumption gives a slightly lower efficiency than the zero-order assumption in the area where they do not coincide (Levenspiel 1989). Thus the first-order assumption should be used in qualitative calculations (which covers most such calculations that would be performed). For ultimate accuracy finite-element calculations should be made.

When calculations of the Theile modulus are possible, it can be used to obtain the effectiveness factor with Equation 6.13. As a general guideline, intraparticle diffusion is not limiting when  $\phi \leq 0.4$  and is strongly limiting when  $\phi \geq 4.0$ . Overall process economics determine the optimal particle size or film thickness. When all the required information is available, the designer can analyze relative transport and reaction rates. The pattern suggested by Levenspiel (1989) offers a clearer view of the significance of surface film resistance for transport from a gas to a catalyst particle for a first-order irreversible reaction:

1. Measure  $v'''$ , the rate of reaction based on volume of particles, mol/[(m<sup>3</sup> solid)·s].
2. Determine whether  $C_{AS}$  needs to be corrected for surface film resistance, that is,

$$\text{Fraction of resistance due to surface film} = \frac{-\nu_A v''' R}{3k_L C_{AL}},$$

which stems from

$$k_L(C_{AL} - C_{AS})S_R = -\nu_A v''' V_P, \quad (6.14)$$

or the rate of transport of A into the surface of particle equals the rate of disappearance of A because of the reaction.

$$C_{AS} = \frac{k_L C_{AL} S_R + \nu_A v''' V_P}{k_L S_R}.$$

Solving for  $C_{AS}$  gives

$$C_{AS} = C_{AL} \left[ 1 - \left( \frac{-\nu_A v''' R}{3k_L C_{AL}} \right) \right]. \quad (6.15)$$

So the term  $(-\nu_A v''' R)/(3k_L C_{AL})$  is a fraction by which  $C_{AL}$  must be reduced to obtain  $C_{AS}$ .

3. Calculate  $C_{AS}$  from Equation 6.15 if justified. Then calculate the Weisz modulus,  $M_W$ , from

$$M_W = \phi^2 \eta_A = \frac{-\nu_A v''' R^2}{9C_{AS} D_e}. \quad (6.16)$$

There is no intraparticle transport resistance if  $M_w \leq 0.15$ ; otherwise, it must be considered.

A slightly different approach may be taken if kinetic and transport characteristics are known. The rate of transport through the surface film is equal to the rate of transport into the particle as given by Fick's law, which in turn is equal to the rate of reaction as determined by using the surface reactant concentration and the particle effectiveness:

$$k_L(C_{AL} - C_{AS})S_p = -\nu_A k'''C_{AS}\eta_p V_p, \quad (6.17)$$

where  $\eta_p$  = effectiveness factor for particle.

An overall efficiency,  $\eta_O$ , taking into account film resistance and intraparticle diffusion resistance, can be defined as

$$\eta_O = \frac{\nu'''}{k'''C_{AL}} \quad (6.18)$$

but

$$\nu''' = k''' \eta_p C_{AS}. \quad (6.19)$$

From Equation 6.17,

$$\begin{aligned} C_{AS} &= \frac{k_L C_{AL} S_p}{k_L S_p - \nu_A k''' \eta_p V_p} \\ &= C_{AL} \frac{1}{1 - \frac{\nu_A k''' R \eta_p}{3k_L}}. \end{aligned} \quad (6.20)$$

Combining Equations 6.18–6.20 gives

$$\eta_O = \frac{\eta_p}{1 - \frac{\nu_A k''' R \eta_p}{3k_L}}. \quad (6.21)$$

Equation 6.21 may be rearranged to obtain

$$\eta_O = \frac{1}{\frac{1}{\eta_p} - \frac{\nu_A k''' R}{3k_L}}. \quad (6.22)$$

Another term,  $\eta_f$ , can be defined as

$$\eta_f = -\frac{3k_L}{v_A k'' R}. \quad (6.23)$$

This term is similar to the gas film effectiveness factor,  $\epsilon_g$ , used by Levenspiel (1989) for gas films around catalyst particles. The expression for the overall effectiveness factor with this new term becomes

$$\eta_O = \frac{1}{\frac{1}{\eta_p} + \frac{1}{\eta_f}}. \quad (6.24)$$

Thus, as discussed by Levenspiel (1989) for gas-catalyst systems, if the reciprocal of effectiveness factor is viewed as resistance, the total is equal to the sum of the parts, with  $\eta_f$  becoming a film effectiveness factor:

$$\frac{1}{\eta_O} = \frac{1}{\eta_p} + \frac{1}{\eta_f}. \quad (6.25)$$

The overall effectiveness factors can be used as modifiers in kinetic calculations for the various types of reactors. These factors allow use of the same types of kinetic analysis used for chemical reaction-limited systems.

## STIRRED-TANK BIOREACTORS

The majority of bioreactors fall into the stirred-tank category. Arbitrarily, nonstirred batch reactors are included as a special case. Analysis of batch systems is covered by the basic equations discussed in Chapter 3, with modifications for mass transfer discussed in the preceding section of this chapter. The batch stirred-tank reactor is a special case of the general category of stirred-tank reactors, having a feed rate of zero. The well-mixed, fluidized bed reactor is usually analyzed as a continuous stirred-tank reactor (CSTR). Stirred-tank bioreactors are referred to by various names and abbreviations. Here the abbreviation CSTR is used whether flow exists or not. Figure 6.1 illustrates schematic flow for a CSTR,

where  $F$  = volumetric feed rate, volume/time;

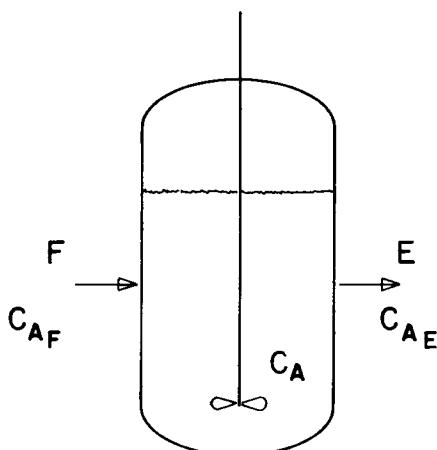
$E$  = volumetric effluent rate, volume/time;

$V_R$  = volume of reaction mixture in reactor;

$C_{AF}$  = concentration of reactant A in feed;

$C_A$  = concentration of reactant A in reactor;

$C_{AE}$  = concentration of reactant A in effluent.



**FIGURE 6.1** Continuous stirred-tank reactor flow scheme.

The reactor is assumed to be in hydraulic steady-state; that is, the volume of the reaction mixture is constant. A further assumption is that there is no volumetric change with reaction. Thus the effluent flow rate,  $E$ , is equal to the feed flow rate,  $F$ . If the reaction mixture is completely mixed, the effluent concentration of A must be equal to that of the reaction mixture; that is,  $C_{AE} = C_A$ . Under these conditions a balance on A yields

$$V_R \frac{dC_A}{dt} = FC_{AF} - FC_A + \nu_A v V_R \quad (6.26)$$

where  $v$  = universal reaction rate, moles stoichiometric conversion/[volume·time].

When the system reaches steady state,

$$\frac{dC_A}{dt} = 0 = \frac{F}{V_R}(C_{AF} - C_A) + \nu_A v. \quad (6.27)$$

The term  $F/V_R$  is called *hydraulic loading* or the *dilution rate*,  $D$ . The reciprocal,  $V_R/F$ , has the units of time. It represents the mean holding time or residence time in the reactor, designated as  $\tau$ . Equation 6.27 is used to determine required reactor size for a given conversion with a fixed enzyme concentration. If, for example, the reaction is first order,

$$\frac{F}{V_R}(C_{AF} - C_A) + \nu_A k_1 C_A = 0 \quad (6.28)$$

or

$$D(C_{A_F} - C_A) + \nu_A k_1 C_A = 0 \quad (6.29)$$

or, solving for required reactor volume,

$$V_R = \frac{F(C_{A_F} - C_A)}{-\nu_A k_1 C_A} \quad (6.30)$$

or, in terms of fractional conversion,  $x$ , of component A,

$$V_R = \frac{F(x - 1)}{\nu_A k_1}. \quad (6.31)$$

The reactor never actually reaches steady state, because an infinite length of time is required, according to Equation 6.26. However, for all practical purposes, the steady-state assumptions may be used. Equation 6.26 may be integrated by using the appropriate rate expression (kinetic and/or mass transport) for rate of reaction. Many free enzyme catalyzed reactions are essentially zero order in reactor startup. For such reactions, Equation 6.26 becomes

$$\frac{dC_A}{dt} = F(C_{A_F} - C_A) + \nu_A k_0 V_R \quad (6.32)$$

or

$$\frac{dC_A}{dt} = D(C_{A_F} - C_A) + \nu_A k_0, \quad (6.33)$$

which, upon integration, yields

$$\ln \left[ \frac{DC_{A_F} + \nu_A k_0 - DC_A}{\nu_A k_0} \right] = -Dt \quad (6.34)$$

or

$$\frac{t}{\tau} = \ln \left[ \frac{\frac{\nu_A k_0}{DC_{A_F}}}{\frac{\nu_A k_0}{DC_{A_F}} + x} \right], \quad (6.35)$$

where  $x = 1 - (C_A/C_{A_F})$ , the fractional conversion of A.

For the steady state, Equation 6.27 may be solved for  $x_s$ , the fractional conversion of A at steady state:

$$D(C_{A_F} - C_A) + \nu_A k_0 = 0;$$

$$D(x_s) = -\frac{\nu_A k_0}{C_{A_F}};$$

$$x_s = 1 + \frac{\nu_A k_0}{DC_{A_F}}. \quad (6.36)$$

Then, stating Equation 6.35 in terms of  $x_s$  from Equation 6.36 gives

$$\frac{t}{\tau} = \ln \left( \frac{x_s}{x_s - x} \right)$$

or

$$\frac{t}{\tau} = \ln \left( \frac{1}{1 - \frac{x}{x_s}} \right). \quad (6.37)$$

If  $a = (x/x_s)$ , or the *fractional approach to steady state*, Equation 6.37 becomes

$$\frac{t}{\tau} = \ln \left( \frac{1}{1 - a} \right). \quad (6.38)$$

Equation 6.38 may be solved to determine the time required for a given approach to steady state or vice versa. For example, at a time  $t$  equal to 3 times the average residence time,  $\tau$ , the conversion of A is 95% of that theoretically attained at steady state. A time of 4.6 times  $\tau$  would be required to reach 99% of steady-state conversion.

For product formation, the steady state with no product in the feed stream is

$$DC_P - \nu_P v = 0, \quad (6.39)$$

where  $C_P$  = concentration of product in effluent;

$\nu_P$  = stoichiometric coefficient for  $P$ , positive for product.

## PLUG-FLOW BIOREACTORS

The plug-flow, or tubular, reactor is used less frequently than the CSTR for biological reactions. It does have some advantages, however, for continuous-flow reaction systems. One obvious advantage is the absence of externally driven mixers, which often are used in stirred-tank reactors. Sealing of such

mixers for preventing contamination can be quite difficult. Problems may develop in mixing and in the addition and removal of gases for the plug-flow reactors, which do not occur in CSTRs.

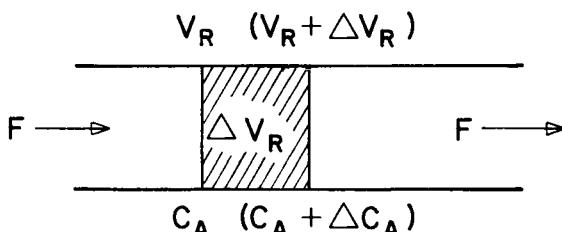
Plug-flow reactors can be used for free enzyme systems as well as for immobilized enzyme systems. The inherent nature of some of the latter (e.g., coated tubes and membranes and trickle-flow systems) fixes them in the plug-flow category (with more or less backmixing). Even fluidized-bed reactors approach plug-flow behavior for the liquid stream when fluid velocity increases beyond the opposing terminal velocity of the suspended particles. Such reactors are called *transport reactors*.

Figure 6.2 illustrates flow through a differential volume element in a hypothetical plug-flow reactor. The assumptions for the basic bioreactor system usually are:

1. Net flow velocity is axially and radially consistent; that is, net flow velocity in the axial direction is the same at any point in the reactor. There is no volumetric expansion or contraction with reaction (unless gases are involved).
2. There is complete mixing in radial directions. Temperatures and concentrations are homogeneous in any plane normal to the flow.

As a consequence of the first assumption, hydraulic flow is constant and equal to the inlet flow,  $F$ . Under these constraints the following balance on component A (mol/t) can be made for a differential volume element,  $\Delta V_R$ , shown in Figure 6.2.

Flow into element	$FC_A$
Production by reaction	$v_{AV}\Delta V_R$
Flow out of element	$F(C_A + \Delta C_A)$
Net balance	$F[C_A - (C_A + \Delta C_A)] + v_{AV}\Delta V_R = 0$ $-F\Delta C_A + v_{AV}\Delta V_R = 0$



**FIGURE 6.2** Differential volume element in a plug-flow reactor with no volume expansion and volumetric feed rate,  $F$ .

Taking limits as  $\Delta V_R$  approaches zero yields

$$-F dC_A + \nu_A v dV_R = 0$$

or

$$dV_R = \frac{F dC_A}{\nu_A v}. \quad (6.40)$$

Equation 6.40 may be integrated with  $C_A$  as the independent variable. For example, if the reaction is first order with respect to A,

$$\int_0^{V_R} dV_R = \frac{F}{\nu_A k_1} \int_{C_{A_F}}^{C_{A_E}} \frac{dC_A}{C_A},$$

$$V_R = \frac{F}{\nu_A k_1} \ln \left( \frac{C_{A_E}}{C_{A_F}} \right), \quad (6.41)$$

or

$$\tau = \frac{1}{\nu_A k_1} \ln (1 - x). \quad (6.42)$$

The term  $\tau$  is equal to the time required to reach the same conversion of A in a batch reactor.

### COMPARISON OF PLUG-FLOW AND CSTR REACTORS

Plug-flow and CSTR reactors may be compared for a sample reaction following Michaelis–Menten kinetics. For the plug-flow reactor, Equation 6.40 becomes

$$dV_R = \frac{F}{\nu_A} \frac{dC_A}{\left( \frac{V_m C_A}{K_{mA} + C_A} \right)}, \quad (6.43)$$

where  $V_m$  = maximum reaction rate in Michaelis–Menten rate law, (moles stoichiometric reaction)/time;

$K_{mA}$  = Michaelis constant.

Separating variables and integrating with respect to  $C_A$  gives

$$\int_0^{V_R} dV_R = \frac{F}{\nu_A V_m} \int_{C_{A_F}}^{C_{A_E}} \left( \frac{K_{mA}}{C_A} + 1 \right) dC_A;$$

or

$$V_R = \frac{F}{\nu_A V_m} \left[ K_{mA} \ln \left( \frac{C_{A_E}}{C_{A_F}} \right) + C_{A_E} - C_{A_F} \right]. \quad (6.44)$$

An equation for the volume of a steady-state CSTR required to accomplish a given conversion with a reaction following Michaelis–Menten kinetics may be derived from Equation 6.27. The equation for volume of required CSTR for any rate law is

$$\frac{F}{V_R} (C_{A_F} - C_A) + \nu_A v = 0 \quad (6.27)$$

or

$$V_R = \frac{F(C_{A_F} - C_{A_E})}{-\nu_A v}. \quad (6.45)$$

Substituting the Michaelis–Menten rate-law expression for  $v$  yields

$$V_R = \frac{F(C_{A_F} - C_{A_E})}{-\nu_A \left( \frac{V_m C_{A_E}}{K_{mA} + C_{A_E}} \right)}. \quad (6.46)$$

Now, the reactor volume required for a plug-flow reactor can be compared with that for a CSTR. Equation 6.44 is used for the plug-flow reactor, and Equation 6.46 is used for the CSTR. Arbitrary parameters for an example comparison are

$$\begin{aligned} F &= 0.1 \text{ L/s}; \\ V_m &= 2.0 \text{ M/(L·s)}; \\ \nu_A &= -1; \\ K_{mA} &= 0.001 \text{ M}; \\ C_{A_F} &= 0.1 \text{ M}; \\ C_{A_E} &= 0.0001 \text{ M}. \end{aligned}$$

With these conditions and kinetic constants (and constant enzyme concentration) the following sizes of reactors are required.

Plug-flow	5.34 mL
CSTR	549 mL

Thus the reactor volume requirement for the CSTR is more than 100 times that for the plug-flow reactor. This comparison is for a reactant conversion of 99.9%. At lower conversions the differences are less dramatic.

## REACTOR COMBINATIONS

The choice of reactors ultimately rests on the actual rate law for the reaction(s) involved. In some cases combinations of reactor types or operating regimes can be justified. The startup of a flow CSTR, for example, can involve a zero flow or batch phase to bring the reactor to desired reactant and product concentrations before commencing actual flow through the reactor. Systems that require stirring—but in which the plug-flow regime might be preferred from a conversion point of view—may optimally consist of CSTRs in series. A plug-flow reactor is approached in theory by an infinite number of infinitesimal CSTRs in series.

# Chapter 7

## Cell Culture Bioreactors

### TYPES OF BIOLOGICAL GROWTH REACTORS

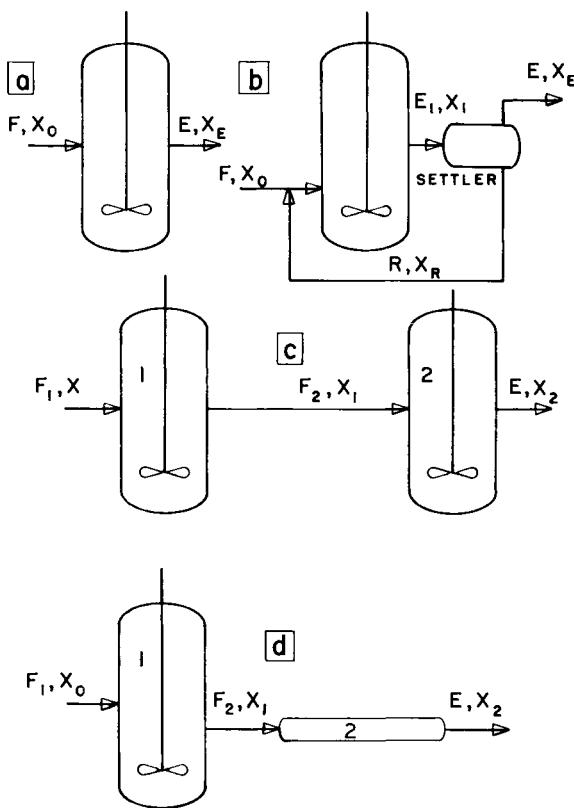
Dispersed-cell bioreactors are often termed single-cell bioreactors. Either the cells multiply in unassociated states, or their growth and activity can be simulated as though that were the case. Proper reactor design for such cultures is dictated by the objectives of the process. Appropriate design for the objective of maximum cell production is not necessarily congruous with that for the objective of optimal substrate conversion or product selectivity. Each system must be analyzed on its own merits and limitations. Any of a large number of possible reactor configurations and operations may be used in cell culture systems. Most, however, are based on either the continuous stirred-tank reactor (CSTR) design or the plug-flow design. As in chemical reactors and enzyme reactors, idealized cases must be corrected for actual behavior. The CSTR's ideal state is defined by the following conditions.

- Reaction mixture is completely mixed.
- Feed is immediately mixed into the reaction mixture.
- Temperature and composition of reaction mixture (cells and medium) are uniform throughout the reactor.
- Product stream has same composition as reaction mixture.

The ideal plug-flow reactor has a *piston flow* in which temperature, flow velocity, and composition (cells and medium) are completely uniform in a plane normal to the bulk flow direction. The ideal batch reactor is a special case of the CSTR in which feed and product flow rates are both zero. The ideal case of the so-called *fed-batch* reactor is a special CSTR in which the product flow rate is zero. Nongrowth cellular systems—as found in many immobilized-cell reactors—can be treated as enzyme systems, even though some may be quite complex because of the presence of enzymes and enzyme complexes for metabolic pathways instead of just one enzymatic reaction.

Biological growth reactors may be operated optimally in a number of different flow arrangements, the choice depending on the particular system and desired performance. Figure 7.1 illustrates some of these arrangements.

The CSTR is the workhorse of biological transformations and growth. It



**FIGURE 7.1** Several typical bioreactor arrangements for biological growth systems:  
 (a) single CSTR; (b) single CSTR with recycle; (c) two CSTRs in series; (d) CSTR in series with plug-flow reactor.

can be operated singly or in series combinations of two or more reactors and with or without cell recycle. The plug-flow biological growth reactor by its nature must have a continuous supply of cells in the feed for sustained operation. This supply may be provided by cell recycle or by insertion of a CSTR ahead of the plug-flow reactor. A plug-flow reactor with a major recycle of reaction mixture is called a *loop reactor*.

### CSTR BIOLOGICAL GROWTH SYSTEMS

The CSTR for biological growth systems is often called the *continuous stirred-tank biological reactor* (CSTBR). Models of the various flows may be devel-

oped, starting with that of the basic flow shown in Figure 7.1(a). Symbols used for this model are

- $F$  = volumetric feed rate, volume/time;
- $E$  = volumetric effluent flow rate, volume/time;
- $X_0$  = concentration of cells in feed, mass or number per unit volume;
- $X$  = concentration of cells in reaction mixture;
- $X_E$  = concentration of cells in effluent;
- $S_0$  = concentration of substrate in feed;
- $S$  = concentration of substrate in reaction mixture;
- $S_E$  = concentration of substrate in effluent;
- $P_0$  = concentration of product in feed (usually zero);
- $P$  = concentration of product in reaction mixture;
- $P_E$  = concentration of product in effluent;
- $V_R$  = volume of reaction mixture.

In the general case, both reaction mixture volume ( $V_R$ ) and composition ( $X, S, P$ ) possibly are variable functions of time. Some simplifying equalities can be stated right away for the ideal case of a completely mixed reactor by definition:

$$X_E = X, \quad S_E = S, \quad \text{and} \quad P_E = P.$$

Then, with the assumption that no reaction mixture specific volume change occurs because of the reactions, a volume balance gives

$$\frac{dV_R}{dt} = F - E. \quad (7.1)$$

Next a balance on cells is made:

$$\begin{aligned} \text{Rate of increase in cell mass} &= (\text{cells in}) - (\text{cells out}) \\ &+ (\text{rate of generation of new cells by multiplication}), \end{aligned} \quad (7.2)$$

or

$$\frac{d(V_RX)}{dt} = FX_0 - EX + V_R \left( \frac{dX}{dt} \right)_{\text{growth}}, \quad (7.3)$$

which yields

$$V_R \frac{dX}{dt} + X \frac{dV_R}{dt} = FX_0 - EX + V_R \left( \frac{dX}{dt} \right)_{\text{growth}}. \quad (7.4)$$

The rate of change of cell concentration then is

$$\frac{dX}{dt} = \frac{F}{V_R} X_0 - \frac{E}{V_R} X + \left( \frac{dX}{dt} \right)_{\text{growth}} - \frac{X}{V_R} \frac{dV_R}{dt}. \quad (7.5)$$

For first-time users, some confusion is likely to develop in the use of Equation 7.5. The following points may aid in understanding:

$\frac{dX}{dt}$  = the net rate of change of concentration of cells in the reaction mixture due to all contributions.

$\left( \frac{dX}{dt} \right)_{\text{growth}}$  = contribution of cell multiplication to the rate of increase of cell concentration. For cultures obeying the Monod rate law, for example, this term becomes

$$\left( \frac{dX}{dt} \right)_{\text{growth}} = \frac{\mu_m S X}{K_S + S}.$$

$\frac{dV_R}{dt}$  = rate of change of total volume of reaction mixture, given by Equation 7.1.

Similar balances can be made on substrate:

$$\frac{dS}{dt} = \frac{F}{V_R} S_0 - \frac{E}{V_R} S + \left( \frac{ds}{dt} \right)_{\text{reaction}} - \frac{S}{V_R} \frac{dV_R}{dt}, \quad (7.6)$$

and on product:

$$\frac{dP}{dt} = \frac{F}{V_R} P_0 - \frac{E}{V_R} P + \left( \frac{dP}{dt} \right)_{\text{reaction}} - \frac{P}{V_R} \frac{dV_R}{dt}. \quad (7.7)$$

These general equations can now be incorporated into models with specific constraints or enhancements for the various CSTR types.

### The Batch Stirred-tank Culture System

A stirred-tank culture system operated without feed or product streams is an ideal batch reactor. It can be treated as an unsteady-state CSTR with both  $F$  and  $E$  equal to zero. Thus the rate of increase in cell population is solely the result of multiplication (and/or death). Hence the expressions for multiplication limited by growth kinetics, substrate supply, or mass transport also are the

culture rate expressions. For example, consider the kinetics for a batch culture following the Monod rate law with first-order cell death. The cell rate of increase is

$$\frac{dX}{dt} = \frac{\mu_m S X}{K_S + S} - k_d X. \quad (7.8)$$

Substrate utilization is given by

$$-\frac{dS}{dt} = q_s X, \quad (7.9)$$

where  $q_s$  = specific substrate consumption rate.

For a culture with an appreciable maintenance factor, then

$$q_s = Y_{S/X} \frac{\mu_m S}{K_S + S} + m. \quad (7.10)$$

The product appearance rate then is

$$\frac{dP}{dt} = q_p X, \quad (7.11)$$

where  $q_p$  = specific product appearance rate.

In the case where there is appreciable nongrowth-associated product synthesis,

$$q_p = Y_{P/X} \frac{\mu_m S}{K_S + S} + B, \quad (7.12)$$

where  $B$  = nongrowth component of specific product appearance rate from Equation 5.12.

Numerical integration may be used to solve Equations 7.8–7.12 for  $S$ ,  $X$ , and  $P$  as functions of time,  $t$ . Simplified cases are solved analytically. For example, the specific multiplication rate,  $\mu$ , may be essentially constant over a range of substrate and cell concentrations. The values of  $Y_{S/X}$  and  $Y_{P/X}$  may also be constant. Further, the maintenance factor,  $m$ , may be negligible. Taking all these simplifications to be valid yields a case that is easily analyzed:

- *Initial conditions*

$$t = 0; \quad X = X_0; \quad S = S_0; \quad \text{and} \quad P = 0$$

- *Rates*

$$\frac{dX}{dt} = \mu X \quad (7.13)$$

$$\frac{dS}{dt} = -Y_{S/X} \frac{dX}{dt} \quad (7.14)$$

$$\frac{dP}{dt} = -Y_{P/S} \frac{dS}{dt} \quad (7.15)$$

- *Conversion stoichiometry*

$$S_0 - S = Y_{S/X}(X - X_0) \quad (7.16)$$

$$P = Y_{P/S}(S_0 - S) \quad (7.17)$$

- *Integral growth*

$$X = X_0 e^{\mu t} \quad (7.18)$$

Combining Equations 7.16 and 7.18 gives

$$\begin{aligned} S &= S_0 + Y_{S/X}(X_0 - X) \\ &= S_0 + X_0 Y_{S/X}(1 - e^{\mu t}) \end{aligned} \quad (7.19)$$

and

$$P = Y_{P/S}(S_0 - S). \quad (7.20)$$

Equation 7.19 may be rearranged to solve for the time required to reach a given substrate concentration:

$$S_0 - S = X_0 Y_{S/X}(e^{\mu t} - 1),$$

$$e^{\mu t} = \frac{S_0 - S}{X_0 Y_{S/X}} + 1,$$

$$\mu t = \ln \left[ \frac{S_0 - S}{X_0 Y_{S/X}} + 1 \right],$$

and

$$t = \frac{1}{\mu} \ln \left[ \frac{S_0 - S}{X_0 Y_{S/X}} + 1 \right]. \quad (7.21)$$

The time required for a given increase in cell population is derived by integration of Equation 7.13:

$$\ln \frac{X}{X_0} = \mu t \quad (7.22)$$

or

$$t = \frac{1}{\mu} \ln \frac{X}{X_0}. \quad (7.23)$$

Note that Equations 7.21 and 7.22 are derived by simple integration and that a limit to  $X$  is imposed by substrate supply. If reaching a substrate concentration near zero under the given constraints were possible, Equation 7.16 would impose a limit on  $X$ :

$$S_0 - 0 = Y_{S/X} (X_{\max} - X_0), \quad (7.24)$$

where  $X_{\max}$  = maximum value of  $X$  under substrate supply limitations.

Then

$$X_{\max} = \frac{S_0}{Y_{S/X}} + X_0. \quad (7.25)$$

Equation 7.24 may be used to determine the minimum value of initial substrate concentration required to produce a given cell population (within limits of validity of initial assumptions):

$$S_0 = Y_{S/X}(X - X_0). \quad (7.26)$$

Determination of batch reactor volume requirement must allow for the following operations between actual growth periods.

- filling with culture medium and adjusting initial conditions of temperature, pH, O<sub>2</sub> concentration, and the like
- adding seed culture
- ...GROWTH PERIOD...
- emptying vessel
- sterilization
- cleaning, changing of air filters, maintenance, and inspection
- sterilization

The ultimate effect of the additional time required for these operations is to increase the reactor size (or number of reactors) required. This effect gives

some incentive for use of continuous systems. However, batch systems have the advantage of producing fewer cell generations per cycle than do continuous-flow CSTR systems. Thus contaminating cells, mycoplasmas, and viruses or undesirable mutant cell strains have less time to develop.

### Fed-batch Cultures

One disadvantage of pure batch culture is that some cultures exhibit substrate inhibition. Another is the impracticality of supplying all the substrate in the beginning culture because of concerns about substrate solubility limits, substrate instability, or culture osmotic pressure. One possible solution for all these problems is to use fed-batch reactors. In fed-batch cultures, feed is added continuously or at intervals. The model developed here is for a fed-batch culture with continuous feed at a variable rate with no removal of product until the end of the culture cycle. For the fed-batch system, Equation 7.1 becomes

$$\frac{dV_R}{dt} = F, \quad (7.27)$$

and Equation 7.5 becomes

$$\frac{dX}{dt} = \frac{F}{V_R} X_0 + \left( \frac{dX}{dt} \right)_{\text{growth}} - \frac{X}{V_R} \frac{dV_R}{dt}. \quad (7.28)$$

Then from Equations 7.27 and 7.28,

$$\frac{dX}{dt} = \frac{F}{V_R} X_0 + \left( \frac{dX}{dt} \right)_{\text{growth}} - \frac{FX}{V_R}. \quad (7.29)$$

Equation 7.6 becomes

$$\begin{aligned} \frac{dS}{dt} &= \frac{F}{V_R} S_0 + \left( \frac{dS}{dt} \right)_{\text{reaction}} - \frac{S}{V_R} \frac{dV_R}{dt} \\ &= \frac{F}{V_R} S_0 + \left( \frac{dS}{dt} \right)_{\text{reaction}} - \frac{FS}{V_R}, \end{aligned} \quad (7.30)$$

and Equation 7.7 becomes

$$\frac{dP}{dt} = \frac{F}{V_R} P_0 + \left( \frac{dP}{dt} \right)_{\text{reaction}} - \frac{FP}{V_R}. \quad (7.31)$$

If the rate-law relationships are known for

$$\left(\frac{dX}{dt}\right)_{\text{growth}}, \quad \left(\frac{dS}{dt}\right)_{\text{reaction}}, \quad \text{and} \quad \left(\frac{dP}{dt}\right)_{\text{reaction}},$$

then analytical or numerical integration can be used with these relationships and Equations 7.27, 7.29, 7.30, and 7.31 to solve for  $X$ ,  $S$ ,  $V_R$ , and  $P$  as functions of time, depending on the flow rate plan set for feed.

The main objective of using the fed-batch system is to control substrate concentration. For this control, relationships may be derived from Equation 7.30 as follows:

$$\frac{dS}{dt} = \frac{F}{V_R}(S_0 - S) + \left(\frac{dS}{dt}\right)_{\text{reaction}}.$$

If

$$\left(\frac{dS}{dt}\right)_{\text{reaction}} = -Y_{S/X}\mu_m \left(\frac{SX}{K_s + S}\right) - mX.$$

Then, setting  $(dS/dt) = 0$  for constant  $S$ ,

$$0 = \frac{F}{V_R}(S_0 - S) - Y_{S/X}\mu X - mX$$

or

$$F = \left(\frac{Y_{S/X}\mu + m}{S_0 - S}\right)XV_R. \quad (7.32)$$

Equation 7.32, in simplified form, is

$$F = CXV_R, \quad (7.33)$$

$$\text{where } C = \left(\frac{Y_{S/X}\mu_m S}{K_s + S} + m\right)\left(\frac{1}{S_0 - S}\right), \quad \text{or} \quad C = \frac{Y_{S/X}\mu + m}{S_0 - S}.$$

The required feed rate then is a simple function of reaction mixture volume and cell concentration. The time relationship for this system is complex, but it can be set up easily for computer control of feed rate without measurement of reaction volume or cell concentration once rate constants have been determined.

### Single Continuous-flow CSTR without Recycle

The continuous-flow CSTR without recycle depicted in Figure 7.1(a) is operated to produce a certain effluent cell concentration or to give a certain substrate conversion or product concentration. In the general case, the system is in hydraulic steady state. That is, the reaction volume,  $V_R$ , is held essentially constant by keeping  $E = F$ , on average. Thus Equation 7.5 becomes

$$\frac{dX}{dt} = \frac{F}{V_R} X_0 - \frac{F}{V_R} X + \left( \frac{dX}{dt} \right)_{\text{growth}}. \quad (7.34)$$

The dilution rate,  $D$ , is

$$D = \frac{F}{V_R}, \quad (7.35)$$

where the units of  $D$  are time<sup>-1</sup>. The reciprocal of  $D$  is  $\tau$ , the mean residence time in the reactor. The general cell mass balance gives

$$\frac{dX}{dt} = D(X_0 - X) + \left( \frac{dX}{dt} \right)_{\text{growth}}. \quad (7.36)$$

If cell concentration,  $X_0$ , in feed is zero,

$$\frac{dX}{dt} = \mu X - DX. \quad (7.37)$$

The rate of change of substrate concentration in general is

$$\frac{dS}{dt} = D(S_0 - S) - Y_{S/X}\mu X. \quad (7.38)$$

At steady state, both  $(dS/dt)$  and  $(dX/dt)$  are zero. Thus from Equation 7.37,

$$0 = \mu X - DX$$

or

$$\mu = D, \quad (7.39)$$

and from Equation 7.38,

$$0 = D(S_0 - S) - Y_{S/X}\mu X$$

or

$$D(S_0 - S) = Y_{S/X}\mu X. \quad (7.40)$$

But  $D = \mu$ , so

$$S_0 - S = Y_{S/X}X. \quad (7.41)$$

When proper flow rates are set to approach steady-state concentrations from some other concentrations, infinite time is required to reach an absolute steady state. A rule of thumb for a CSTR with constant feed rate is that at least three complete reactor turnovers (three times the residence time,  $\tau$ ) should be allowed to ensure close approach to steady state. Usually, start-up involves some operation without flow until the desired cell concentration is reached in the reactor.

If  $\mu$  is not a function—direct or indirect—of  $X$ , then  $X$  is indeterminate at steady state. Thus control setting  $D = \mu$  does not provide control of  $X$ . According to Equation 7.37,  $X$  decreases if  $D > \mu$  and increases if  $D < \mu$ . Hence  $X$  can be controlled by varying  $D$ . Exercise of this option in control distinguishes the *turbidostat* from the *chemostat*. In the turbidostat, cell density is measured generally by light absorption, which indicates turbidity. This turbidity reading is controlled by adjusting feed rate, or  $D$ . This adjustment allows control of cell concentration in the unstable control regime encountered in log-phase growth. In the chemostat, dilution rate is held constant. The dilution rate must be less than  $\mu_m$ , the maximum specific growth rate, for a stable steady state to exist. If  $D$  is equal to  $\mu_m$ , then  $X$  is indeterminate, and a slight imbalance can lead to loss of the cell population. The culture is in an unstable control region. If  $D > \mu_m$ , then  $X$  diminishes until it reaches zero. This occurrence is termed *washout*. It can happen in balanced chemostat systems when environmental stresses or toxic substances cause an extended reduction in  $\mu$  below  $D$ . This situation is demonstrated graphically in the next section.

Equations 7.39 and 7.41 both must be satisfied in CSTR design. Obviously, cells cannot multiply when substrate is completely depleted, so an absolute limit to cell multiplication is set by stoichiometry, as expressed by Equation 7.41, when  $S \approx 0$ . The kinetic limit expressed by Equation 7.39, with  $\mu$  as a function of  $S$ , generally controls before complete conversion of substrate can be attained. A CSTR flow system based on Monod kinetics is

$$\begin{aligned} \mu &= \mu_m \frac{S}{K_S + S}; \\ S &= S_0 - Y_{S/X}X; \\ D &= \mu = \mu_m \frac{(S_0 - Y_{S/X}X)}{K_S + S_0 - Y_{S/X}X}; \\ S_0 &= \frac{\mu_m Y_{S/X}X + K_s D - D Y_{S/X}X}{\mu_m - D}; \end{aligned} \quad (7.42)$$

where  $S_0$  = required concentration of substrate in feed to produce a concentration of cells,  $X$ , in the product stream.

### Graphic Analysis of Single and Multiple CSTRs

The general equation for a CSTR in a series of CSTRs at steady state can be used to solve for the required dilution rate (from Equation 7.36):

$$D(X_n - X_{n-1}) = r_x, \quad (7.43)$$

where  $r_x = \left( \frac{dX}{dt} \right)_{\text{growth}}$ ;

$X_n$  = cell concentration in reactor  $n$ ;

$X_{n-1}$  = cell concentration in feed to reactor  $n$ .

Examination of Equation 7.43 shows that a plot of  $r_x$  versus  $X$  can be used to solve for the required dilution rate:

1. Draw a line beginning at point  $(X_{n-1}, 0)$  and intersecting the  $r_x$  versus  $X$  curve at  $X_n$ .
2. Determine  $D$  as the slope of the line.

Alternatively,  $X_n$  can be determined:

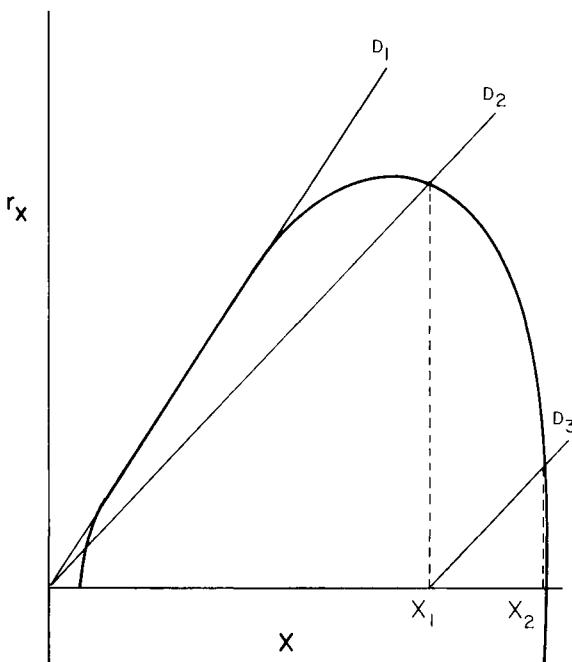
1. Draw a line originating at point  $(X_{n-1}, 0)$  with slope =  $D$ .
2. Find  $X_n$  as the value of  $X$  at the intersection of the line with the  $r_x$  versus  $X$  curve.

These graphic constructions are shown in Figure 7.2. For the first in a series of CSTRs, if there are no cells in the feed:

$$X_{n-1} = X_0 = 0.$$

The dilution lines thus begin at the origin. The line for dilution rate  $D_1$  intersects with the  $r_x$  versus  $X$  curve in the log-phase region. The solution is not a single point, but a line. That is, any value of  $X$  along this intersection would be in the set of solutions for  $X$ . The line for  $D_2$ , which is smaller than  $D_1$ , has two intersections with the  $r_x$  versus  $X$  curve. The first intersection usually represents a trivial solution for  $X$ , because it is in a growth phase generally associated with the lag phase of a batch culture. Data for this phase for  $r_x$  are usually time dependent and not meaningful for a long-term steady-state culture. The best approach generally is to develop the rate data from continuous steady-state cultures rather than batch cultures because of such disparities.

The slope of the  $r_x$  versus  $X$  curve has a value equal to the specific growth



**FIGURE 7.2** Graphic constructions for CSTR designs:  $r_x$  = rate of cell multiplication,  $dX/dt$ ;  $X$  = cell concentration. Straight lines have slope equal to dilution rate,  $D$ .

rate,  $\mu$ . Even though this slope goes from positive to negative, beyond the log phase, the second derivative is always negative (Figure 7.2). Consequently, any decrease in  $X$  gives a higher  $\mu$ , and an increase in  $X$  causes a decrease in  $\mu$ . Thus the population is self-correcting for any transient changes in itself, and  $D$  remains constant. Similarly, changes in  $D$  lead to establishment of new, stable steady states.

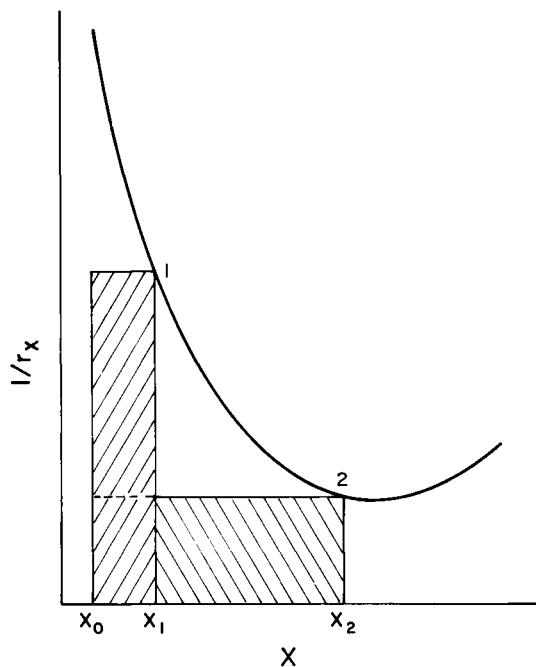
These procedures can be extended to calculations for CSTRs in series, such as in Figure 7.1(c). Ordinarily, such reactor arrangements are made up of reactors of equal volume with an equal volumetric flow rate through all reactors. Therefore the dilution lines all have the same slope. The sample solution for two CSTRs in series illustrated in Figure 7.2 is for dilution rate  $D_2$  in reactor 1 and dilution rate  $D_3$  in reactor 2.

Another type of graphic analysis involves a slightly different application of Equation 7.34. This general equation, as applied to a CSTR in a series at steady state, becomes

$$V_{R_n} = \frac{F(X_n - X_{n-1})}{r_x}, \quad (7.44)$$

where  $V_{R_n}$  = volume of reactor  $n$ .

If  $F = 1.0$ , then  $V_r = \text{volume per unit feed rate}$ , or simply  $\tau$ . Equation 7.44 indicates that required reactor volume can be determined quite simply when the relationship between  $r_x$  and  $X$  is known. The most convenient graph to use is a plot of  $(1/r_x)$  versus  $X$  as illustrated in Figure 7.3. It shows two alternative reactor designs for reaching a cell concentration  $X_2$  with a feed cell concentration  $X_0$ . The first alternative is to choose a two-reactor configuration, with reactor 1 producing a cell concentration  $X_1$  and reactor 2 producing the final cell concentration  $X_2$ . The volume of reactor 1, from Equation 7.44, is represented by the rectangular area  $(X_1 - X_0) \times (1/r_x)_1$ . The volume of reactor 2 is represented by the rectangular area  $(X_2 - X_1) \times (1/r_x)_2$ . The second alternative involves the use of only one reactor. The volume requirement for a single reactor is represented by the rectangular area  $(X_2 - X_0) \times (1/r_x)_2$ . The total volume requirement clearly is less for the single reactor than for the two-reactor combination. The two-reactor combination for this situation would be chosen only if volume restraints were imposed on a single reactor by physical limits or mass-transfer or mixing limits. If total volume were the only consideration, the choice would be a single CSTR until some point beyond the



**FIGURE 7.3** Graphic analysis of CSTRs in series. Solid curve represents growth rate data for  $(1/r_x)$  growth.

minimum point of the  $(1/r_x)$  versus  $X$  curve is reached. Here, as discussed later, the choice might be a CSTR followed by a plug-flow reactor.

### PLUG-FLOW REACTORS FOR CELL GROWTH

The ideal plug-flow reactor is one in which distributions of all reactants and products are completely uniform in a plane normal to bulk flow. When two phases are involved, this condition means no slippage between the two flowing phases. However, in gas–liquid systems—even when the gas velocity may be higher than the liquid velocity—the liquid phase can be modeled by using plug-flow assumptions, with some adjustments, in most cases.

The advantages of the plug-flow reactor—simplicity of construction, absence of mechanical seals, and minimal volume requirement for first-order chemical conversions—have led to many attempts to culture cells in such reactors. Some have been successful, others unsuccessful. Continuing development of techniques and mechanical designs leads to changing guidelines in choice of CSTR versus plug-flow configurations.

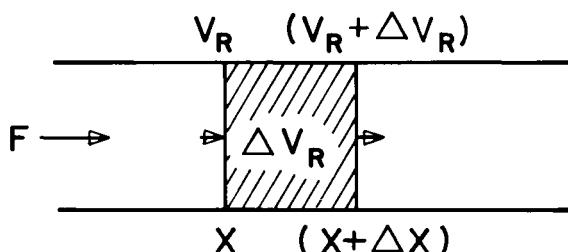
The ideal plug-flow model has a differential volume element, as illustrated in Figure 7.4. A material balance on cells for the differential volume  $\Delta V_R$  gives

$$FX + r_x \Delta V_R = F(X + \Delta X)$$

or

$$r_x \Delta V_R = F \Delta X,$$

where  $r_x = \left( \frac{dX}{dt} \right)_{\text{growth}}$ .



**FIGURE 7.4** Differential element of plug-flow cell culture reactor. Volumetric feed rate,  $F$ , is assumed constant for all elements (no volume change with reactions),  $V_R$  = reactor volume, and  $X$  = cell concentration.

Taking limits as  $\Delta V_R$  approaches zero yields

$$\begin{aligned} \lim_{\Delta V_R \rightarrow 0} r_x \Delta V_R &= \lim_{\Delta X \rightarrow 0} F \Delta X; \\ r_x dV_R &= F dX; \\ \frac{dV_R}{F} &= \frac{dX}{r_x}; \end{aligned} \quad (7.45)$$

$$\begin{aligned} \int_0^{V_R} dV_R &= F \int_{X_0}^X \frac{dX}{r_x}; \\ V_R &= F \int_{X_0}^X \frac{dX}{r_x} \end{aligned} \quad (7.46)$$

or

$$\frac{V_R}{F} = \tau = \int_{X_0}^X \frac{dX}{r_x}; \quad (7.47)$$

where  $\tau$  = residence time in reactor.

Equation 7.47 shows that the residence time,  $\tau$ , is equal to the time,  $t$ , required to achieve the same cell concentration increase in a batch reactor. Equation 7.45 is a differential form of the CSTR equation, Equation 7.43. The integral forms, Equations 7.46 and 7.47, are equivalent to solutions for an infinite array of infinitesimal CSTRs in series, as demonstrated for enzyme reactors. An analytical solution for the integral of the right-hand side of Equation 7.47 involves a system for which the cell multiplication rate is described by a logistic equation (Chapter 5):

$$r_x = \mu_m X \left( 1 - \frac{X}{X_t} \right). \quad (5.8)$$

Equation 7.47 becomes

$$\tau = \frac{1}{\mu_m} \int_{X_0}^X \frac{dX}{X \left( 1 - \frac{X}{X_t} \right)}, \quad (7.48)$$

which, upon integration, yields

$$\tau = \frac{1}{\mu_m} \left( \ln \frac{X}{X_0} - \frac{1}{X_t} \ln \frac{X_t - X}{X_t - X_0} \right). \quad (7.49)$$

No solution exists if  $X_0 = 0$ , which is easily deduced intuitively. If there are no cells in the feed, they obviously cannot multiply. Cells can be supplied to the feed as fresh inoculum. More likely sources are recycled product stream or effluent from a CSTR ahead of the plug-flow reactor.

In cases where substrate conversion is of primary interest, the use of models utilizing substrate concentration as the variable in place of cell population may be more desirable. The relationships developed are similar to those for batch reactors, as can be demonstrated with a system in which cell multiplication is first order and substrate utilization is exclusively growth-associated. First, consider a batch system under these constraints.

If  $X_0$  = initial cell concentration and  $S_0$  = initial substrate concentration,

$$\begin{aligned} X &= X_0 + \frac{S_0 - S}{Y_{S/X}}, \\ \frac{dX}{dt} &= \mu X, \\ \frac{dS}{dt} &= \frac{dS}{dx} \cdot \frac{dX}{dt} \\ &= -Y_{S/X}\mu \left( X_0 + \frac{S_0 - S}{Y_{S/X}} \right), \\ \mu \int_0^t dt &= - \int_{S_0}^S \frac{dS}{X_0 Y_{S/X} + S_0 - S}, \end{aligned}$$

and

$$t = \frac{1}{\mu} \ln \left( \frac{X_0 Y_{S/X} + S_0 - S}{X_0 Y_{S/X}} \right). \quad (7.50)$$

Then, for a plug-flow reactor with  $V_R$  as the independent variable,

$$\begin{aligned} \frac{dS}{dV_R} &= \frac{dS}{dX} \cdot \frac{dX}{dV_R}, \\ &= -Y_{S/X} \cdot \frac{r_X}{F}, \\ &= \frac{-Y_{S/X}}{F} \mu X, \\ &= -\frac{Y_{S/X}}{F} \mu \left( X_0 + \frac{S_0 - S}{Y_{S/X}} \right), \end{aligned}$$

$$\frac{\mu}{F} \int_0^{V_R} dV_R = - \int_{S_0}^S \frac{dS}{X_0 Y_{S/X} + S_0 - S},$$

and

$$V_R = \frac{F}{\mu} \ln \left( \frac{X_0 Y_{S/X} + S_0 - S}{X_0 Y_{S/X}} \right), \quad (7.51)$$

or

$$\tau = \frac{1}{\mu} \ln \left( \frac{X_0 Y_{S/X} + S_0 - S}{X_0 Y_{S/X}} \right). \quad (7.52)$$

Thus a comparison of Equations 7.50 and 7.52 shows that the time required to reach a given substrate conversion in a batch reactor is the same as the residence time required in a plug-flow reactor for the same conversion. These sample solutions are for relatively simply mathematical relations. More complex systems require solution by numerical integration. Equation 7.52 is used to determine substrate concentration as a function of residence time. Solving for  $S$  gives

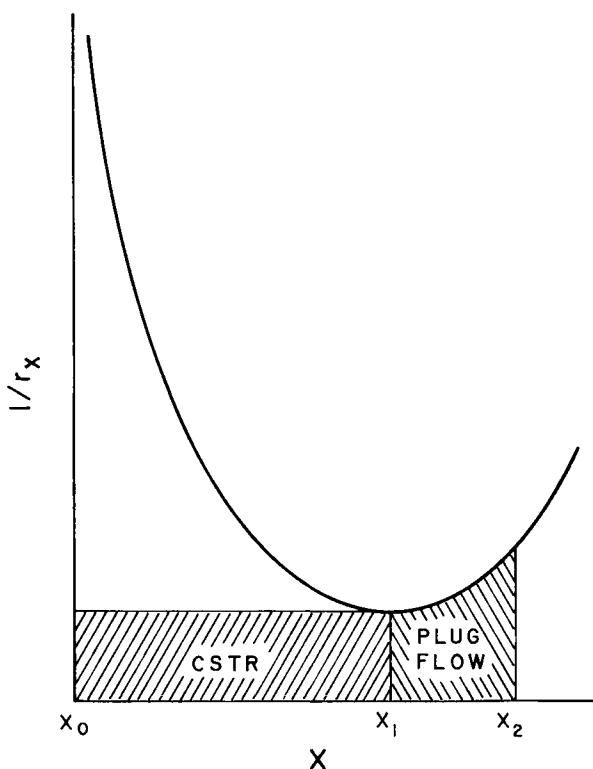
$$1 + \frac{S_0 - S}{X_0 Y_{S/X}} = e^{\mu\tau},$$

$$S_0 - S = (e^{\mu\tau} - 1) X_0 Y_{S/X},$$

or

$$S = S_0 - X_0 Y_{S/X} (e^{\mu\tau} - 1). \quad (7.53)$$

Equation 7.47 may be solved graphically for  $\tau$ , or volume per unit feed rate:  $\tau$  is the area under the  $(1/r_x)$  versus  $X$  curve from  $X_{n-1}$  to  $X_n$ . Even though more sophisticated analytic or numeric methods may be used to solve for residence time in plug-flow reactors or CSTRs, the graphic solution allows a visual appreciation of the nuances involved. Bischoff (1966) used graphic methods to illustrate the size considerations involved in choice of reactor configurations. Figure 7.5 shows a final graphic solution for the minimum volume requirement for the nonrecycle reactor configuration required to produce a cell content of  $X_2$ , beginning with feed having a low cell content. As demonstrated in the preceding section, the minimum volume requirement for a CSTR in the region where the slope of the  $(1/r_x)$  versus  $X$  curve is negative is given by one CSTR. The value of  $\tau$  for that CSTR is indicated by the shaded rectangle from  $X_0$  to  $X_1$ . The  $\tau$  required for a plug-flow reactor to go from  $X_0$  to  $X_1$  would be larger, or equivalent to all the area under the  $(1/r_x)$  versus  $X$



**FIGURE 7.5** Graphic illustration of minimum-volume reactor configuration;  $X$  is at the minimum point of  $(1/r_x)$ . Adapted from K. B. Bischoff (1966). (Reproduced with permission from the author and the Canadian Society for Chemical Engineering.)

curve from  $X_0$  to  $X_1$  (infinite if  $X_0$  is actually zero). However, in the region from  $X_1$  to  $X_2$ , the  $\tau$  required for a CSTR is the rectangular area  $(X_2 - X_1) \times (1/r_x)_2$ . In comparison, the  $\tau$  required for a plug-flow reactor is the area under the curve from  $X_1$  to  $X_2$ . Thus in the region where the  $(1/r_x)$  versus  $X$  curve has a positive slope, the plug-flow reactor is preferred. The combination of a CSTR followed by a plug-flow reactor shown in Figure 7.5 is the configuration that gives the minimum total volume requirement.

## RECYCLE OPERATIONS

Many cell culture systems utilize cell recycle. With plug-flow reactors, the requirement for cells in feed can be satisfied by use of cell recycle. With CSTRs, cell recycle can be used to maximize cell population in the reactors while maintaining log-phase growth. In the specialized area of waste treatment, recycle of cells in the form of activated sludge is used both to maximize

conversion of waste material and to minimize production of activated sludge. With regard to the latter, sludge produced in aerated phases of the process is broken down in anaerobic phases.

Figure 7.1(b) illustrates a CSTR with a settler for cell separation and recycle. Relative values of cell concentrations in product and recycle can be controlled by settler operation. If cells are not desired in product, a cell product stream also may be produced. As mentioned before, the usual purpose of recycle in a CSTR is to increase the active cell population in a culture. If increasing cell population is accompanied by decreasing activity because of death or crowding, that purpose is defeated. There is an optimum cell population for each situation. The amount of medium recycled in the recycle stream depends on design and operation of the settler. For simplicity here, the amount of recycled medium is taken as negligible. Appreciable recycled medium dilutes the culture. In addition, recycled medium contains any toxic materials formed during growth. Therefore—especially in multiple-CSTR or plug-flow reaction systems designed to minimize product inhibition—maximum removal of medium from recycle becomes important.

Some general equalities for the recycle system in Figure 7.1(b), assuming steady-state operation, must first be stated. The volumetric input equals output, or

$$F = E. \quad (7.54)$$

Then

$$E_1 = F + R, \quad (7.55)$$

where  $E_1$  = volumetric effluent flow rate from reaction vessel;  
 $R$  = volumetric recycle flow rate.

Mass balance on cells is

$$FX_0 + V_R\mu X_1 = FX_E, \quad (7.56)$$

where  $X_1$  = concentration of cells in reactor;  
 $V_R$  = volume of reactor;  
 $\mu$  = specific growth rate of cells.

If  $X_0 = 0$ , the case for most cell cultures,

$$FX_E = V_R\mu X_1 \quad (7.57)$$

and

$$D = \frac{F}{V_R} = \mu \frac{X_1}{X_E}. \quad (7.58)$$

*Note:* If there were a cell product stream, with volumetric flow rate =  $F_W$  and cell concentration,  $X_R$ , Equation 7.57 would be

$$FX_E + F_W X_R = V_R \mu X_1 \quad (7.59)$$

or

$$V_R = \frac{FX_E + F_W X_R}{\mu X_1}. \quad (7.60)$$

If  $X_E = 0$ ,

$$\frac{F_W}{V_R} = \mu \frac{X_1}{X_R}. \quad (7.61)$$

Equation 7.58 can be expressed in terms of cell recycle ratio. If  $\beta$  = fraction of cells in product recycled,

$$RX_R = \beta FX_1. \quad (7.62)$$

Then, with  $X_0 = 0$ , the cell mass balance gives

$$\beta FX_1 + V_R \mu X_1 = FX_1,$$

$$F(1 - \beta) = V_R \mu,$$

and

$$D = \frac{F}{V_R} = \frac{\mu}{1 - \beta}. \quad (7.63)$$

The equations become more lengthy when substrate dependence of  $\mu$ , effect of substrate and product content of recycle, product inhibition, and the like are added.

The value of recycle in maintaining the stability and efficiency of waste treatment systems is generally accepted. One disadvantage is that recycled sludge consisting of lower concentrations of live and active micro- and macro-organisms is less effective in removing phosphate and nitrogen from the waste stream. In systems other than waste treatment, the positive value of recycle in maintaining high cell population and thus requiring a smaller reactor may be outweighed by the required investment and operating costs, as well as possible

contamination resulting from the settler addition. Also, the older cells present in recycle systems may produce undesirable products in addition to having a higher likelihood of mutation. Therefore recycle may not always be quite so desirable as it might first appear.

## **CONTINUOUS CULTURE OF PHOTOSYNTHETIC ORGANISMS**

The modeling of continuous culture systems for production of high concentrations of photosynthetic organisms requires application of growth-rate equations corrected for light intensity as a function of depth in the medium. Otherwise, the principles are the same as those discussed for culture bioreactors in general. A summary of one type of model for a CSTR chemostat to culture algae on a continuous flow basis has been presented (Bader, 1984). It provides an overview of the type of attack required for analysis and simulation of such systems.

Bader used the Tamiya model for the growth rate of algae in dense cultures. This model differs from the Heifetz and Quinlan model presented in Equation 5.65. The final model used by Bader for cell growth rate is

$$\mu X = \left( \frac{dX}{dt} \right)_{\text{growth}} = \frac{\mu_m}{\epsilon W} \ln \left( \frac{\mu_m + \alpha I_0}{\mu_m + \alpha I_0 e^{-\epsilon XW}} \right), \quad (7.64)$$

where  $X$  = cell concentration;

$\mu_m$  = maximum specific cell growth rate;

$I_0$  = incident light intensity;

$\alpha$  = constant;

$\epsilon$  = extinction coefficient for cells;

$W$  = thickness of culture (depth).

The constants are determined from batch culture data as follows:

1. Using dilute batch cultures where the light intensity on all cells is essentially  $I_0$  and

$$\mu = \frac{1}{X} \left( \frac{dX}{dt} \right)_{\text{growth}} \approx \frac{\mu_m \alpha I_0}{\mu_m + \alpha I_0}, \quad (7.65)$$

determine  $\alpha$  and  $\mu_m$  from the Lineweaver–Burk plot of  $1/\mu$  versus  $1/I_0$ , which gives the slope as  $1/\alpha$  and the  $y$  intercept as  $1/\mu_m$ .

2. Determine  $(dX/dt)_{\text{growth}}$  for high concentrations of cells at a constant value of  $I_0$ . Under these conditions, light absorption is high, and  $e^{-\epsilon XW}$  can be assumed to be zero for approximation purposes. Then

$$\left(\frac{dX}{dt}\right)_{\text{growth}} = \frac{\mu_m}{\epsilon W} \ln\left(1 + \frac{\alpha I_0}{\mu_m}\right). \quad (7.66)$$

Determine  $\epsilon$  from the observed values of  $(dX/dt)_{\text{growth}}$  as

$$\epsilon = \frac{\frac{\mu_m}{\mu_m}}{\left(\frac{dX}{dt}\right)_{\text{growth}}} \frac{\ln\left(1 + \frac{\alpha I_0}{\mu_m}\right)}{W}. \quad (7.67)$$

Then the general mass balance for cells for a CSTR with no cells in feed becomes

$$\frac{dX}{dt} = \frac{\mu_m}{\epsilon W} \ln\left(\frac{\mu_m + \alpha I_0}{\mu_m + \alpha I_0 e^{-\epsilon X W}}\right) - \frac{F}{V_R} X, \quad (7.68)$$

where  $V_R$  = reactor volume.

At steady state,  $(dX/dt) = 0$ , and the allowable dilution rate is

$$D = \frac{\mu_m}{\epsilon X W} \ln\left(\frac{\mu_m + \alpha I_0}{\mu_m + \alpha I_0 e^{-\epsilon X W}}\right). \quad (7.69)$$

Equation 7.68 may be solved by iteration.

## STERILIZATION OF CULTURE MEDIA

The topic of sterilization is very broad, and complete coverage would require a book in itself. Here, just the basics of cell killing in batch or plug-flow reactions are explored. The simplest approach to cell killing is to view death as a simple first-order disappearance reaction with Arrhenius-type temperature dependence. Then

$$\frac{dN}{dt} = -A e^{-(E_a/RT)} N, \quad (7.70)$$

where  $N$  = total number of live cells;

$E_a$  = activation energy;

$R$  = universal gas constant;

$T$  = absolute temperature.

Upon integration, Equation 7.70 yields

$$N = N_0 \exp \left[ -A \int_{t=0}^t e^{-(E_a/RT)} dt \right], \quad (7.71)$$

where  $N_0$  = initial number of live cells.

The target for sterilization is to reduce the probability of having one of the most resistant organisms in its spore form to  $10^{-3}$  in the whole fermentor or preparation. The time requirement is then a function of size of the system. Integration of Equation 7.71 by numeric or graphic methods to determine the time requirement. Example magnitudes of the constants would be

$$A = 1.14 \times 10^{-42} \text{ min}^{-1}, \quad \text{and} \quad E_a = 2.87 \times 10^5 \text{ J/(mol K).}$$

These are approximate values for spores of *Bacillus stearothermophilus* and should be used for example calculation purposes only.

## SCALE-UP OF CELL-CULTURE BIOREACTORS

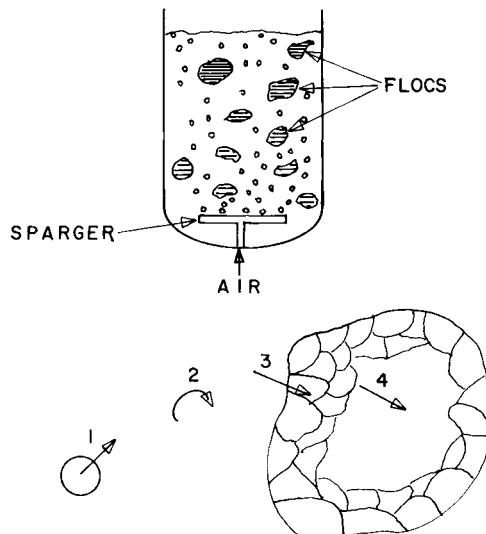
The topic of scale-up of cell-culture bioreactors also includes scale-down. *Scale-up* is the procedure involved in determining the design for a reactor of one throughput from experimental data gained from a reactor operating at a different capacity. It is a process that involves trial-and-error, the amount depending on the type of reactor involved. Considerations for a fluidized-bed, immobilized-cell reactor are quite different from those for a single-cell, air-lift reactor, for example. Scale-up of cell culture systems in many cases is limited by cell frailty and physiological requirements not encountered in ordinary scale-up of physical systems. In scale-up, the engineering objective is usually to maintain dimensional similarity as size is increased. Degree of mixing and heat-transfer coefficients, for example, might be maintained by keeping Reynolds numbers constant in some cases. In many cases for bioreactors, the objective must be to maintain mass-transfer coefficients for oxygen and carbon dioxide. The specific difficulties arise when one objective cannot be met without conflicting with the other.

A plethora of literature on bioreactor scale-up has been produced during the past few years. Much of the literature has concerned the scale-up problems for highly aerobic systems, such as those used for hydrocarbon-utilizing yeasts and those now used extensively for culture of molds for antibiotic production. The mold cultures have complications not found in most single-cell systems in that the mycelia are more sensitive to shear from mixers and that the cultures are non-newtonian and difficult to model. More detailed discussions are presented by Blanch and Dunn (1974), Ho *et al.* (1987), Hubbard (1987), Kafarov

*et al.* (1987–1988), Kargi and Moo-Young (1985), Mooyman (1987), Moo-Young and Blanch (1981), Oldshue (1987), and Trilli (1986), among others.

Some of the mass-transfer constraints for scale-up are discussed after consideration of the simple culture system illustrated in Figure 7.6. Initial laboratory studies of a mold culture requiring a high air input—but with unknown shear sensitivity—might be to carry out runs utilizing a reactor, as shown. For a batch culture, the primary transport processes would be those shown and the following: transfer of carbon dioxide to gas bubbles, bubble formation at the sparger, coalescence and breakup of bubbles, escape of bubbles at the surface of the medium, formation and breakup of flocs, transport of nutrients into and out of cells, heat transfer within and out of flocs, heat transfer to or from the medium at the reactor walls and liquid surface, and evaporation of volatile components of the medium into bubbles and to gas at the surface. In a flow system, macromixing becomes a further important variable. It usually is quantified in terms of mixing time, that is, the time required for essentially complete mixing of a tracer into the culture medium.

Data from laboratory runs with the simple configuration might indicate that a higher oxygen transfer rate is required. It might be accomplished by changing the reactor configuration to a deep-tube air-lift or inclusion of more spargers. In most cases it would be accomplished by incorporating a mixer into the system. Moo-Young and Blanch (1981) showed that the highest rate of transfer can generally be obtained from the mixer for a given power input. For



**FIGURE 7.6** Mixed-tank reactor for aerobic culture of floc-forming cells. Transport of oxygen is illustrated in four steps: (1) transfer from air bubble to fluid; (2) macromixing within fluid; (3) transfer from fluid to floc; and (4) transfer into body of floc.

illustrative purposes here, the assumption is that this high oxygen transfer is needed and that the final laboratory design includes a mixer.

The most common configuration for a mixed-tank reactor is that of a vertical cylinder. The first thought in retaining similarity of different sizes is to retain the same height-to-diameter ratio,  $H/D$ . Physical similarity would then dictate that the mixer impeller diameter be increased in proportion to the increase in reactor diameter to maintain similarity. This adds to problems already present in the nature of the scale-up. If the scaling factor,  $S$ , is defined as  $D_2/D_1$ , the ratio of the larger reactor diameter to the smaller, the following scale factors result:

Reactor volume	$S^3$
Top surface to volume	$S^{-1}$
Wall area to volume	$S^{-1}$
Impeller velocity (constant angular velocity)	$S$

Most of the authors mentioned have attacked and discussed the mixing problems in such systems. Trilli (1986) for example showed relationships for scale-up of the following derived parameters (at constant angular velocity of impeller):

Specific power	$S^2$
Mixing time	$S^{1/6}$

Specific power is power input to the mixer per unit reactor volume ( $P/V_R$ ). The mentioned authors have summarized some of the mixed-tank correlations for  $k_{La}$  for oxygen transfer. The correlations generally contain this specific power raised to some power. Thus scale-up attempts might intuitively first be aimed at maintaining constant specific power. With sensitive mycelial structures, this objective is impossible if impeller similarity is to be maintained. Constant specific power can be maintained by decreasing the impeller rotation speed,  $N$ . However, as summarized by Trilli (1986), the following relationships exist:

$$\begin{aligned} \frac{P_0}{V_R} &\propto N^3 D_i^2, \\ t_m &\propto N^{-2/3} D_i^{1/6}; \\ s &\propto ND_i; \end{aligned}$$

where  $D_i$  = impeller diameter;

$s$  = impeller tip speed;

$t_m$  = mixing time.

Therefore maintaining constant specific power means that

$$\frac{N_2}{N_1} = \left( \frac{D_{i1}}{D_{i2}} \right)^{2/3}$$

or

$$\frac{S_2}{S_1} = \left( \frac{D_{i2}}{D_{i1}} \right)^{1/3}$$

Thus impeller tip speed would increase by the scale factor raised to the one-third power. If the laboratory unit were already being operated at the limit of 12–14 m/s impeller tip speed (Trilli 1986), maintaining constant specific power would not be practical. Other solutions, such as multiple impellers or mixers, would have to be found. At any rate, an increase in size usually means increased mixing time, more nonideal CSTR mixing, and coincident nonideality of reactor performance. Consequently, increasing trial reactor sizes in relatively small steps for unknown systems is prudent.

# List of Symbols

---

## ENGLISH

$a$	$[A]_0 + [P]_0$ for reversible reaction, M
$a$	primary reactant concentration
$a$	empirical constant in Equation 4.12
$a$	interfacial area
$a$	fractional approach to steady state, $x/x_s$
$a_i$	number of gram-atoms of hydrogen per C-mole of component $I$
$b$	empirical constant in Equation 4.12
$b_i$	number of gram-atoms of oxygen per C-mole of component $I$
$A$	frequency factor in Arrhenius equation
$A, [A]$	concentration of reactant A, M
$A_d$	Arrhenius preexponential factor for cell death, time <sup>-1</sup>
$[A]_{eq}$	equilibrium concentration of reactant A, M
$[A]_0$	concentration of reactant A at $t = 0$ , M
$B$	cell-mass related production factor, time <sup>-1</sup>
$B$	constant in Equation 5.23
$[B]$	concentration of reactant B, M
$[B]_0$	concentration of reactant B at $t = 0$ , M
$c$	constant in Equation 3.30, K <sup>-1</sup> s <sup>-1</sup>
$c$	empirical constant in Equation 4.12
$c_{A,G}$	concentration of A in bulk gas phase, mol/cm <sup>3</sup>
$c_{A,i,G}$	concentration of A in gas phase at gas–liquid interface, mol/cm <sup>3</sup>
$c_{A,i,L}$	concentration of A in liquid phase at gas–liquid interface, mol/cm <sup>3</sup>
$c_{A,L}$	concentration of A in bulk liquid phase, mol/cm <sup>3</sup>
$c_{O_2,L}^*$	concentration of oxygen in liquid given by Henry's law or related equilibrium equation or data for equilibrium with bulk gas phase, mol/cm <sup>3</sup>
$C$	concentration (or solubility in concentration units), mol/L
$C$	integration constant
$C$	constant in Equation 7.33
$C_A$	concentration of A
$C_{AB}$	concentration of A in bulk fluid
$C_{AE}$	concentration of A in effluent
$C_{AF}$	concentration of reactant A in feed
$C_{AL}$	concentration of A in liquid phase

$C_{AS}$	concentration of A at radius $R$ (solid side of surface)
$C_{ASL}$	concentration of A at liquid interface with particle
$C_i$	inhibitor concentration
$C_{CR_{O_2}}$	critical oxygen concentration
$C_i^*$	limiting inhibitor concentration
$C_{O_2}^*$	saturation oxygen concentration in liquid for given oxygen partial pressure in gas phase
$C_P$	concentration of product
$\Delta C_p$	heat capacity change for stoichiometric reaction, $J/(mol \cdot K)$
$C_{p_i}$	heat capacity of stream $I$
$C1$	$O_2$ concentration in water, $mL(STP)/L$
$C2$	$O_2$ concentration in water, mass ppm
$d$	empirical constant in Equation 4.12
$d$	density of a mycelial pellet
$D$	heat dissipation rate, $kW$
$D$	dilution rate, $F/V_R$ , time $^{-1}$
$D_{AB}$	diffusivity (or diffusion coefficient) of A in medium B, $cm^2/s$
$D_e$	effective diffusivity of A in medium or pores, $m^2/s$
$D_i$	impeller diameter
$e$	empirical constant in Equation 4.12
$e_o$	total enzyme concentration
$E$	reactor effluent rate, volume/time
$E_A$	activation energy in Arrhenius equation
$E_{aA}$	concentration of enzyme adsorbed on amorphous cellulose
$E_{aC}$	concentration of enzyme adsorbed on crystalline cellulose
$E_d$	activation energy for cell death rate equation
$[E]_d$	stoichiometric concentration of denatured enzyme active centers, mol/L
$[E]_n$	stoichiometric concentration of normal (undenatured) enzyme active centers, mol/L
$[E]_t$	active enzyme concentration at time $t$
$[E]_0$	stoichiometric concentration of enzyme active centers
$[E]_0$	total enzyme concentration
$f$	empirical constant in Equation 4.12
$F$	reactor feed rate, volume/time
$F_n$	volumetric feed rate to reactor $n$
$F_W$	volumetric rate for cell product stream
$FE(\%)$	fermentation efficiency, Equation 5.16
$g$	empirical constant in Equation 4.12
$g$	maximum value of cell population, $X$ , mass or number per unit volume
$g$	local acceleration of gravity
$\Delta g_{av/e}^\circ$	average Gibbs free energy change per mole of electrons transferred in substrate conversion to products, $kJ/mol$

$\Delta g_{ci}^\circ$	standard-state Gibbs free energy of combustion of component I, kJ/C-mole
$\Delta g_{CN}^\circ$	standard-state Gibbs free energy of combustion of nitrogen source to yield N <sub>2</sub> , kJ/mol
$\Delta G^\circ$	Gibbs free-energy change for stoichiometric reaction at biological standard state and system temperature, J/mol
$\Delta G_1^\circ$	standard-state Gibbs free-energy change for reaction 1
$G_1$	glucose concentration
$G_2$	cellobiose concentration
$G_{2A}$	concentration of amorphous cellulose
$G_{2C}$	concentration of crystalline cellulose
Gr	Grashof number, Equation 4.13
$h$	Hill coefficient
$h$	Planck constant, J·s
$\Delta h_{ci}^\circ$	standard-state heat of combustion of component <i>I</i> , kJ/C-mole
$h_i$	enthalpy of stream <i>I</i> , kJ/mol
$\Delta H^\circ$	standard-state enthalpy change, J/mol
$\Delta H^\circ'$	enthalpy change at biological standard state
$\Delta H^\ddagger$	enthalpy of activation, J/mol
$\Delta H_0^\ddagger$	enthalpy of activation at zero pressure
$i$	inhibitor concentration
$I$	light intensity
$I$	inhibitor concentration
$I_0$	incident light intensity
$I_t$	threshold, or compensation, light intensity
[I]	concentration of substance I, M
$J_{Az}$	molar flux of A in the <i>z</i> direction, relative to bulk flow, mol/(cm <sup>2</sup> ·s)
$k$	general rate constant
$k$	constant in Equation 5.55
$k''$	first-order reaction rate constant giving rate in (moles stoichiometric reaction)/(m <sup>3</sup> ·s)
$k$	Boltzmann constant, J/K
$k_A$	specificity constant for A, L/(mol·s)
$k_B$	specificity constant for B, L/(mol·s)
$k_{AB}$	reciprocal Dalziel coefficient for A and B
$k_A^Z$	reciprocal Dalziel coefficient
$k_c$	gas concentration mass-transfer coefficient, cm/s
$k_d$	specific reaction rate constant for enzyme deactivation
$k_d$	specific death rate constant, time <sup>-1</sup>
$k_H$	Henry's law constant, units of pressure
$k_L$	liquid concentration mass-transfer coefficient, cm/s
$k_x$	molar liquid-phase mass-transfer coefficient, mol/(cm <sup>2</sup> ·s)
$k_y$	molar gas mass-transfer coefficient, mol/(cm <sup>2</sup> ·s)

$k_0$	zero-order reaction-rate constant
$k_o$	catalytic constant, $s^{-1}$
$k_o^{\text{app}}$	apparent value of $k_o$ , catalytic constant
$k_1$	first-order reaction-rate constant
$k_4, k_5$	rate constants for Equations 3.49 and 3.50
$K$	reaction equilibrium constant for simplest stoichiometric equation yielding one mole of product, units variable
$K$	Monod-type constant, Equation 5.65, units of light intensity
$K_a$	acid dissociation constant
$K_{a1}$	acid dissociation constant for first ionization
$K_{a2}$	acid dissociation constant for second ionization
$K_e$	equilibrium constant
$K_H$	constant for solubility of $\text{CO}_2$ in aqueous solutions, $\text{mol}/(\text{L}\cdot\text{atm})$
$K_i$	inhibition constant
$K_{iA}$	substrate inhibition constant
$K_{ic}$	competitive inhibition constant for $I$
$K_{iu}$	uncompetitive inhibition constant for $I$
$K_{i,4}$	cellobiose inhibition constant
$K_{mA}$	Michaelis constant for A, mol/L
$K_{ma}$	Michaelis constant for conversion of component a, units of concentration
$K_{mB}$	apparent Michaelis constant for B
$K'_{mA}$	specific Michaelis constant for substrate inhibition
$K_S$	Monod constant for substrate, concentration units
$K_S$	constant in Equation 5.21
$K_S$	constant in Equation 5.22
$K_{S\text{eff}}$	effective modified form of Monod constant, Equation 5.37
$K_T$	equilibrium constant at temperature $T$
$K_W$	$[\text{H}^+] [\text{OH}^-]$
$K_1$	equilibrium constant for enzyme denaturation
$K_1$	constant in Haldane equation, Equation 3.10, $\text{M}^{-1}\text{s}^{-1}$
$K_1$	equilibrium constant for simplest integral stoichiometric equation for enzyme heat denaturation
$K_1$	first acid dissociation constant
$K_1$	constant in Equation 5.66
$K_2$	constant in Equation 5.66
$K_2$	second acid dissociation constant
$K_2$	constant in Haldane equation, Equation 3.10
$K_3, K_4$	constants in Haldane equation, Equation 3.10, $\text{M}^{-1}$
$l$	length
$L$	characteristic length of system
$m$	maintenance coefficient, time $^{-1}$
$m$	empirical parameter between $-1$ and $\infty$ , but not 0, in Equation 5.20
$m$	constant in Han-Levenspiel equations

$M_B$	molecular weight of solvent B
$M_W$	Weisz modulus, Equation 6.16
$n$	number of streams
$n$	order of reaction
$n$	constant in Han-Levenspiel equations
$N$	number of cells
$N$	mixing impeller rotation speed, rotations/time
$N_{AR}$	flux of A in radial direction at radius = $R$
$N_{Az}$	molar flux of A in the $z$ direction with respect to fixed coordinates, mol/(cm <sup>2</sup> ·s)
$N_0$	cell population at time zero
$p_i$	partial pressure of component $i$
$p$	absolute pressure, kPa or atm, as specified
$P$	mechanical power input, kW
$P_i$	pressure of stream $i$ , kPa absolute, unless otherwise specified
$P_o$	reactor outlet pressure, kPa
$P_t$	total system pressure
$[P]_0$	initial concentration of product, mol/L
$P_0$	mixing power input
$\text{Pe}$	Péclet number, Equation 4.17
$pK_a$	$-\log_{10} K_a$
$pK_H$	$-\log_{10} K_H$
$pK_W$	$-\log_{10} K_W$
$q_P$	specific product appearance rate, Equation 7.11
$q_S$	specific substrate consumption rate, Equation 5.13
$Q$	rate of heat production, kW
$r$	radius
$r_P$	volumetric product formation rate, moles or mass per unit volume per unit time
$r_p$	radius of pellet or particle
$r_S$	volumetric substrate consumption rate, moles or mass per unit volume per unit time
$r_X$	rate of growth of cells, mass or number per unit volume per unit time
$R$	universal gas constant, 8.314 J/(mol K), unless otherwise specified
$R$	equivalent radius of particle or slab
$R$	volumetric recycle flow rate
$\text{Re}$	Reynolds number, Equation 4.14
$s$	impeller tip speed, length/time
$S$	substrate concentration
$S$	surface area of element normal to diffusion of A
$S$	reactor scaling factor
$S$	salinity, parts per thousand by mass
$\Delta S^\circ$	entropy change at biological standard state
$S_1$	salinity, $S/(34.4 \text{ mass } [\%])$ , parts per thousand

$\Delta S^\ddagger$	entropy of activation, J/(mol K)
$S$ , $[S]$	substrate concentration
$S^*$	limiting substrate concentration
$S_E$	concentration of substrate in effluent
$S_p$	surface area of pellet or particle
$S_p$	surface area of slab
$S_R$	outer surface area of particle
$S_0$	concentration of substrate in feed
$S_0$	concentration of substrate at time zero
$S_1$	concentration of substrate 1
$S_2$	concentration of substrate 2
$Sc$	Schmidt number, Equation 4.15
$Sh$	Sherwood number, Equation 4.11
$t$	time
$t_d$	doubling time
$t_m$	mixing time
$T$	temperature, K
$T_i$	temperature of stream $I$ , K
$T_o$	reactor outlet temperature, K
$u$	fluid velocity, cm/s
$u_{mz}$	net molar velocity of mixture in $z$ direction, cm/s
$v$	reaction rate, (moles stoichiometric reaction)/(m <sup>3</sup> ·s)
$v_i$	molar volume of stream $I$ at temperature $T_i$ and pressure $P$ , m <sup>3</sup> /mol
$v_m$	reaction rate at optimal pH
$v'''$	reaction rate based on volume of reactor, (moles stoichiometric reaction)/(m <sup>3</sup> ·s)
$V$ , $V_m$	limiting reaction rate, (concentration)/time
$\Delta V^\ddagger$	molar volume change of activation, m <sup>3</sup> /mol
$V'$	constant in Equation 3.17
$V_A$	solute A molar volume at normal boiling point, cm <sup>3</sup> /mol
$V_p$	volume of pellet or particle
$V_p$	volume of slab
$V_R$	volume of reaction mixture in reactor
$V_s$	volume of solid
$VP_i$	vapor pressure of pure component $I$ at system temperature
$w$	thickness of peripheral growth zone in a mycelial pellet
$W$	yield constant, Equation 5.25
$W$	thickness of culture (depth)
$x$	fractional conversion
$x$	mole fraction in liquid phase
$x_A$	mole fraction of A in bulk liquid phase
$x_{A,i}$	mole fraction of A in liquid phase at gas–liquid interface
$x_i$	mole fraction of component $I$ in the liquid phase
$x_s$	fractional conversion of reactant at steady state

$X$	cell concentration, mass or number per unit volume
$X$	mass of a mycelial pellet
$X^*$	limiting cell concentration
$X_E$	concentration of cells in effluent
$X_{\max}$	maximum cell concentration under substrate supply limitation
$X_p$	mass of active peripheral zone in mycelial pellet
$X_R$	concentration of cells in recycle
$X_t$	terminal cell concentration
$X_0$	concentration of cells in feed
$X_0$	concentration of cells at time zero
$X_n$	concentration of cells in outlet of reactor $n$
$X_n$	concentration of cells in reactor $n$
$Y_A$	mole fraction of A in bulk gas phase, mol/cm <sup>3</sup>
$Y_{A,i}$	mole fraction of A in gas phase at gas–liquid interface
$Y_{\text{ave}}$	cell yield, grams dry mass per mole of electrons transferred in substrate reaction to yield products
$Y_{P/S}$	ratio of amount of product produced to amount of substrate consumed, mass/mass or mole/mole
$Y_{P/X}$	ratio of mass of product produced per mass of cells appearing
$Y_{S/X}$	ratio of mass of substrate converted per mass of cells produced
$Y_{X/S}$	ratio of mass of cells produced per mass of substrate converted
$z$	distance in $z$ direction
$z$	approach to equilibrium, $[A] - [A]_{\text{eq}}$ , for reversible reaction, M
$z_1$	initial value of $z$ , M
$z_t$	value of $z$ at time $t$ , M
$[Z]$	concentration of product Z, M

## GREEK

$\alpha_i$	coefficient of $[A]^i$ in numerator of rational rate expression, $M^{(1-i)}s^{-1}$
$\beta$	fraction of cells in product recycled
$\beta_i$	coefficient of $[A]^i$ in denominator of rational rate expression, $M^{-1}$
$\gamma_i$	degree of reduction of component $I$ or activity coefficient as specified in text
$\epsilon$	extinction coefficient for cells, Equation 7.64
$\eta_A$	effectiveness factor, Equation 6.11
$\eta_B$	viscosity of solvent B, cp
$\eta_f$	film effectiveness factor, Equation 6.23
$\eta_O$	overall efficiency, Equation 6.18
$\eta_P$	effectiveness factor for particle, Equation 6.17
$\eta_{th}$	thermodynamic efficiency
$\lambda$	constant in Equation 5.22
$\tau$	residence time in reactor

$\mu$	Arrhenius activation energy, J/mol
$\mu$	fluid viscosity, g/(cm·s)
$\mu$	specific growth rate, time <sup>-1</sup>
$\mu_m$	maximum specific growth rate constant, time <sup>-1</sup>
$\mu_{m\text{eff}}$	effective modified form of maximum specific growth rate constant, $\mu_m$ , Equation 5.38
$\mu_o$	uninhibited value of $\mu_m$ , time <sup>-1</sup>
$\nu$	specific product formation rate, Equation 5.14
$\nu$	kinematic viscosity, Equation 4.16, cm <sup>2</sup> /s
$\nu_i$	stoichiometric coefficient of reactant or product, positive for products and negative for reactants
$\rho$	liquid density
$\Delta\rho$	liquid density – gas density
$\tau$	residence time
$\phi$	association factor of solvent B, dimensionless
$\phi$	Thiele modulus, Equation 6.12
$\phi_H$	heat production rate, kJ/s
$\phi_i$	vector rate for stream $I$ into a reactor, positive for input and negative for output, kJ/mol
$\phi_N$	net rate of flow of nitrogen source into system, mol/s
$\phi_O$	net uptake rate of O <sub>2</sub> , mol/s
$\phi_s$	net rate of substrate flow into system, C-mole/s
$\phi_x$	net rate of cell flow out of system, C-mole/s

# REFERENCES

---

- Aiba, S., Humphrey, A.E., and Millis, N.F. (1973). *Biochemical Engineering*, 2nd ed. University of Tokyo Press, Tokyo.
- Andrews, J.F. (1968). "A Mathematical Model for the Continuous Culture of Micro-organisms Utilizing Inhibitory Substrates." *Biotechnol. Bioeng.*, 10:707-723.
- Atkinson, B. (1979). "Biochemical Reaction Engineering." In Coulson, J.M., et al. (editors), *Chemical Engineering*, vol. 3, pp. 367-424. Pergamon Press, Oxford.
- Bader, F.G. (1984). "Application of Reaction Kinetics to Algal Growth." In Crynes, B.L., and Fogler, H.S. (editors), *AIChEMI Modular Instruction: Series E, Kinetics*, pp. 27-33. American Institute of Chemical Engineers, New York.
- Bailey, J.E. (1980). "Biochemical Reaction Engineering and Biochemical Reactors." *Chem. Eng. Sci.* 35:1854-1886.
- Bailey, J.E., and Ollis, D.F. (1986). *Biochemical Engineering Fundamentals*, 2nd ed. McGraw-Hill, New York.
- Battino, R., Rettich, T.R., and Tominaga, T. (1983). "The Solubility of Oxygen and Ozone in Liquids." *J. Phys. Chem. Ref. Data*, 12:163-182.
- Benson, B.B. and Krause, D., Jr. (1976). "Empirical laws for dilute aqueous solutions of nonpolar gases." *J. Chem. Phys.* 64:689-709.
- Bird, R.B., Stewart, W.E., and Lightfoot, E.N. (1960). *Transport Phenomena*. John Wiley & Sons, New York.
- Bischoff, K.B. (1966). "Optimal Continuous Fermentation Design." *Can. J. Chem. Eng.*, 44:281-284.
- Blanch, H.W., and Dunn, I.J. (1974). "Modelling and Simulation in Biochemical Engineering." In Ghose, T.K., Fiechter, A., and Blakebrough, N. (editors), *Advances in Biochemical Engineering*, vol. 3, pp. 127-165. Springer-Verlag, New York.
- Brown, S.W., Oliver, S.G., Harrison, D.E.R., and Righelato, R.C. (1981). "Ethanol Inhibition of Yeast Growth and Fermentation: Differences in the Magnitude and Complexity of the Effect." *Eur. J. Appl. Microbiol. Biotechnol.*, 11:151-155.
- Butler, J.N. (1991). *Carbon Dioxide Equilibria and Their Applications*. Lewis Publishers, Chelsea, Michigan.
- Chao, K.C., and Greenkorn, R.A. (1975). *Thermodynamics of Fluids*. Marcel Dekker, New York.
- Cleland, W.W. (1970). "Steady State Kinetics." pp. 1-65. In Boyer, P.D. (editor), *The Enzymes*, vol II, 3rd ed. Academic Press, New York.
- Dixon, M., and Webb, E.C. (1979). *Enzymes*, 3rd ed. Longman Group, London.
- Edsall, J.T., and Gutfreund, H. (1983). *Biothermodynamics, The Study of Biochemical Processes at Equilibrium*. John Wiley & Sons, Chichester, England.
- Fernandez-Moran, H. et al. (1965). "Electron Microscopic and Biochemical Studies of Pyruvate Dehydrogenase Complex of *Escherichia coli*." *Science*, 145:930-932.

- Forage, R.G., Harrison, D.E.F., and Pitt, D.E. (1985). "Effect of Environment on Microbial Activity." In Moo-Young, M., Bull, A.T., and Dalton, H. (editors), *Comprehensive Biotechnology*, Vol. 1, pp. 251–280. Pergamon Press, Oxford.
- Fredrickson, A.G. (1983). "Interactions of Microbial Populations in Mixed Culture Situations." pp. 201–227. In Blanch, H.W., Papoutsakis, E.T., and Stephanopoulos, G. (editors), *Foundations of Biochemical Engineering, Kinetics and Thermodynamics in Biological Systems, ACS Symposium Series No. 207*. American Chemical Society, Washington, D.C.
- Gaden, E.L. (1959). "Fermentation Process Kinetics." *J. Biochem. Microb. Tech. Eng.*, 1:413–429.
- Haldane, J.B.S. (1965). *Enzymes*. M.I.T. Press, Cambridge, Massachusetts.
- Han, K., and Levenspiel, O. (1988). "Extended Monod Kinetics for Substrate, Product, and Cell Inhibition." *Biotechnol. Bioeng.*, 32:430–437.
- Heifetz, P.B., and Quinlan, A.V. (1988). "Evaluation of the Effect of Light Intensity on the Rate of Photo Biomass Production by the Blue-green Alga *Spirulina platensis* in Semicontinuous Suspension Culture." In Dean, R.C., Jr., and Nerem, R.M. (editors), *Bioprocess Engineering Colloquium*, pp. 57–61. American Society of Mechanical Engineers, New York.
- Henley, J.P., and Sadana, A. (1984). "A Mathematical Analysis of Enzyme Stabilization by a Series-Type Mechanism: Influence of Chemical Modifiers." *Biotechnol. Bioeng.*, 26:959–969.
- Ho, C.S., Baddour, R.F., and Stalker, M.J. (1987). "The Oxygen Transfer Coefficient in Aerated Stirred Reactors and Its Correlation with Oxygen Diffusion Coefficients." In Ho, C.S., and Oldshue, J.Y. (editors), *Biotechnology Processes, Scale-up and Mixing*, pp. 85–95. American Institute of Chemical Engineers, New York.
- Holt, R.A., Cairns, A.J., and Morris, J.G. (1988). "Production of butanol by *Clostridium puniceum* in batch and continuous culture." *Appl. Microbiol. Biotechnol.*, 27:319–324.
- Holtzapple, M.T., Caram, H.S., and Humphrey, A.E. (1984). "The HCH-1 Model of Enzymatic Cellulose Hydrolysis." *Biotech. Bioeng.*, 26:775–780.
- Hoppe, G.K., and Hansford, G.S. (1982). "Ethanol Inhibition of Continuous Anaerobic Yeast Growth" *Biotechnol. Lett.*, 4:39–44.
- Howell, J.A., Chi, C.T., and Pawlowsky, U. (1972). "Effect of Wall Growth on Scale-Up Problems and Dynamic Operating Characteristics of the Biological Reactor." *Biotechnol. Bioeng.*, 14:253–265.
- Hubbard, D.W. (1987). "Scale-up Strategies for Bioreactors." In Ho, C.S., and Oldshue, J.Y. (editors), *Biotechnology Processes, Scale-up and Mixing*, pp. 168–184. American Institute of Chemical Engineers, New York.
- Humphrey, A.E. (1986). Biotechnology Lectures, The University of Texas at Austin.
- Humphrey, A.E., et al. (1977). "Production of Single Cell Protein from Cellulose Wastes." *Biotech. Bioeng. Symp.*, 7:45–64.
- International Union of Pure and Applied Chemistry (IUPAC). (1981). "Symbolism and Terminology in Chemical Kinetics, 1980." *Pure Appl. Chem.*, 53:753–771.
- Johnson, F.H., Eyring, H., and Stover, B.J. (1974). *The Theory of Rate Processes in Biology and Medicine*. John Wiley & Sons, New York.
- Jones, R.P. (1987). "Measures of Yeast Death and Deactivation and Their Meaning." *Process Biochem.*, 22:118–127, 130–134.
- Kafarov, V.V., Vinarov, A. Yu., and Gordeev, L.S. (1987–1988). "Modeling of bioreactors." *Internat. Chem. Eng.*, 27:615–641 (1987) and 28:14–35 (1988).

- Kargi, F., and Moo-Young, M. (1985). "Transport Phenomena in Bioprocesses." In Moo-Young, M., Cooney, C.L., and Humphrey, A.E. (editors), *Comprehensive Biotechnology*, Vol. 2, pp. 5–56. Pergamon Press, Oxford.
- Kolker, Y. (1987). "A Piecewise-Linear Growth Model: Comparison with Competing Forms in Batch Culture." *J. Math. Biol.*, 25:543–551.
- Laidler, K.J., and Bunting, P.S. (1973). *The Chemical Kinetics of Enzyme Action*. Clarendon Press, Oxford.
- Levenspiel, O. (1989). *The Chemical Reactor Omnibook*. Oregon State University Bookstores, Corvallis, Oregon.
- Lewis, G.N., and Randall, M. (1961) [Rev. by Pitzer, K.S., and Brewer, L.]. *Thermodynamics*, 2nd ed. McGraw-Hill, New York.
- Mahler, H.R., and Cordes, E.H. (1971). *Biological Chemistry*, 2nd ed. Harper & Row, New York.
- Mandenius, C.F., et al. (1988). "Kinetic Models for Enzymic Cellulose Degradation in Aqueous Two-Phase Systems." *Biotech. Bioeng.*, 31:203–207.
- Matsché, N.F., and Andrews, J.F. (1973). "A Mathematical Model for the Continuous Cultivation of Thermophilic Microorganisms." *Biotechnol. Bioeng. Symp.* no. 4, pp. 77–90.
- Mooyman, J.G. (1987). "Scaling Oxygen Mass Transfer in Agitated Fermentors." *Biotechnol. Bioeng.* 29:180–186.
- Moo-Young, M., and Blanch, H.W. (1981). "Design of Biochemical Reactors, Mass Transfer Criteria for Simple and Complex Systems." *Adv. in Biochem. Eng.*, 19:1–69.
- Moo-Young, M., and Blanch, H.W. (1983). "Kinetics and Transport Phenomena in Biological Reactor Design." In Blanch, H.W., Papoutsakis, E.T., and Stephanopoulos, G. (editors), *Foundations of Biochemical Engineering, Kinetics and Thermodynamics in Biological Systems* (ACS Symposium Series 207), pp. 335–354. American Chemical Society, Washington, D.C.
- Munjal, P. and Stewart, P.B. (1971). "Correlation Equation for Solubility of Carbon Dioxide in Water, Seawater, and Seawater Concentrates." *J. Chem. Eng. Data*, 16:170–307.
- Nagodawithana, T.W., and Steinkraus, K.H. (1976). "Influence of the Rate of Ethanol Production and Accumulation on the Viability of *Saccharomyces cerevisiae* in 'Rapid Fermentation.'" *Appl. Environ. Microbiol.*, 31(2):158–162.
- Navarro, J.M., and Durand, G. (1978). "Fermentation Alcoolique, Influence de la Température sur l'accumulation d'Alcool dans les Cellules de Levure." *Ann. Microbiol. (Paris)*, 129b:215–224.
- Netter, H. (1959). *Theoretische Biochemie, Physikalisch-Chemische Grundlagen der Lebensvorgänge*. Springer-Verlag, Berlin.
- Nomenclature Committee of the International Union of Biochemistry (NC-IUB). (1982). "Symbolism and Terminology in Enzyme Kinetics, Recommendations 1981." *Eur. J. Biochem.*, 128:281–291.
- Novak, M., Strehaino, P., Moreno, M., and Goma, G. (1981). "Alcoholic Fermentation: On the Inhibitory Effect of Ethanol." *Biotechnol. Lett.*, 4:39–44.
- Oldshue, J.Y. (1987). "Current Situation in Fluid Mixing for Fermentation Processes." In Ho, C.S., and Oldshue, J.Y. (editors), *Biotechnology Processes, Scale-up and Mixing*, pp. 3–5. American Institute of Chemical Engineers, New York.
- Ollis, D.F. (1983). "Kinetics of Enzyme Systems." In Blanch, H.W., Papoutsakis, E.T., and Stephanopoulos (editors), *Foundations of Biochemical Engineering, Kinetics*

- and Thermodynamics in Biological Systems* (ACS Symposium Series No. 207), pp. 27–52. American Chemical Society, Washington, D.C.
- Panchal, C.J., and Stewart, G.G. (1980). "The Effect of Osmotic Pressure on the Production and Excretion of Ethanol and Glycerol by a Brewing Yeast Strain." *J. Inst. Brew.* 86:207–210.
- Parsons, R.V. (1984). *Efficiency and Kinetics of Ethanol Production by Saccharomyces cerevisiae*. Master's thesis, University of Calgary, Department of Chemical and Petroleum Engineering.
- Parsons, R.V., McDuffie, N.G., and Din, G.A. (1984). "pH Inhibition of Yeast Ethanol Fermentation in Continuous Culture." *Biotechnol. Lett.*, 6:677–80.
- Pearl, R., and Reed, L.J. (1920). "On the rate of growth of the population of the United States since 1790 and its mathematical representation." *Proc. Natl. Acad. Sci. USA*, pp. 275–288.
- Pfeil, W. (1986). "Unfolding of Proteins." In Hinz, H.J. (editor), *Thermodynamic Data for Biochemistry and Biotechnology*, pp. 349–376. Springer-Verlag, Berlin.
- Pirt, S.J. (1967). "A Theory of the Mode of Growth of Fungi in the Form of Pellets in Submerged Culture." *Proc. Roy. Soc. London Ser., B*, 166:369–373.
- Pirt, S.J. (1975). *Principles of Microbe and Cell Cultivation*. Blackwell Scientific, Oxford.
- Prokop, A. (1983). "Reactor Design Fundamentals." In Blanch, H.W., Papoutsakis, E.T., and Stephanopoulos, G. (editors), *Foundations of Biochemical Engineering, Kinetics and Thermodynamics in Biological Systems* (ACS Symposium Series 207), pp. 355–376. American Chemical Society, Washington, D.C.
- Reid, R.C., Prausnitz, J.M., and Poling, B.E. (1987). *The Properties of Gases and Liquids*, 4th ed. McGraw-Hill, New York.
- Righelato, R.C. (1975). "Growth Kinetics of Mycelial Fungi." In Smith, J.E., and Berry, D.R. (editors). *The Filamentous Fungi, Vol. I of Industrial Mycology*, pp. 79–103. Edward Arnold, London.
- Righelato, R.C., Rose, D., and Westwood, A.W. (1981). "Kinetics of Ethanol Production by Yeast in Continuous Culture." *Biotechnol. Lett.*, 3:3–8.
- Roels, J.A. (1983a). *Energetics and kinetics in biotechnology*. Elsevier Biomedical Press, Amsterdam.
- Roels, J.A. (1983b). "Macroscopic Thermodynamics and the Description of Growth and Product Formation in Microorganisms." In Blanch, H.W., Papoutsakis, E.T., and Stephanopoulos, G. (editors), *Foundations of Biochemical Engineering, Kinetics and Thermodynamics in Biological Systems* (ACS Symposium Series 207), pp. 295–322. American Chemical Society, Washington, D.C.
- Ryu, D.D.Y. et al. (1982). "Effect of Compression Milling on Cellulose Structure and on Enzymatic Hydrolysis Kinetics." *Biotechnol. Bioeng.*, 24:1047–1067.
- Sadana, A., and Henley, J.P. (1987). "Single-Step Unimolecular Non-First-Order Enzyme Deactivation Kinetics." *Biotechnol. Bioeng.*, 30:717–723.
- Schügerl, K. (1987). *Bioreaction engineering. Reactions involving microorganisms and cells*, vol 1. John Wiley & Sons, New York.
- Slater, J.H. (1985). "Microbial Growth Dynamics." In Moo-Young, M., Cooney, C.L., and Humphrey, A.E. (editors), *Comprehensive Biotechnology*, vol. 2, pp. 189–213. Pergamon Press, Oxford.
- Stainer, R.Y., Adelberg, E.A., and Ingraham, J. (1976). *The Microbial World*, 4th ed. Prentice-Hall, Englewood Cliffs, New Jersey.
- Stouthamer, A.H., and Van Verseveld, H.W. (1985). "Stoichiometry of Microbial

- Growth." In Moo-Young, M., Bull, A.T., and Dalton, H. (editors), *Comprehensive Biotechnology*, Vol. 1, pp. 215-238. Pergamon Press, Oxford.
- Thomas, D.S., and Rose, A.H. (1979). "Inhibitory Effect of Ethanol on Growth and Solute Accumulation by *Saccharomyces cerevisiae* as Affected by Plasma-Membrane Lipid Composition." *Arch. Microbiol.*, 122:49-55.
- Treybal, R.E. (1980). *Mass-Transfer Operations*. 3rd ed. McGraw-Hill, New York.
- Trilli, A. (1986). "Scale-Up of Fermentations." In Demain, A.L., and Solomon, N.A. (editors), *Manual of Industrial Microbiology and Biotechnology*, pp. 277-307. American Society for Microbiology, Washington, D.C.
- Verhulst, P.F. (1839). "Notice sur la que la population suit dans son accroissement." *Corr. Math. Phys. Publ. Para. A.*, 10:113-121.
- Wald, S., Wilke, C.R., and Blanch, H.W. (1984). "Kinetics of the Enzymatic Hydrolysis of Cellulose." *Biotechnol. Bioeng.*, 26:221-230.
- Weiss, R.F. (1970). "The solubility of nitrogen, oxygen and argon in water and sea-water." *Deep-Sea Research*, 17:721-735.

# INDEX

---

- Acid-base equilibrium, 7  
Acids, weak, 7  
Aerobic growth, 9, 10  
Algae, 75  
Amensalism in mixed cultures, 73  
Antagonism in mixed cultures, 73  
Arrhenius equation, cell death, 62, 115     enzymatic reactions, 31  
Anaerobic growth, 10  
Autotrophic growth, 74
- Bacteriophage, 73, 74  
Bases, weak, 6  
Bioreactor, 2  
Bioreactors for cell culture, 93     batch, 52, 96  
          chemostat, 103  
          combinations, 104, 110  
          comparisons, 109  
          CSTR, 73, 94, 102  
          dilution rate, 102  
          residence time, 102, 106  
          steady state, 102  
          substrate requirement, 103  
          volume, 102, 105  
fed-batch, 100  
graphical analysis, 104  
loop, 94  
plug-flow, 93, 107  
plug-flow residence time, 108  
photosynthetic growth, 114  
recycle, 111  
scale-up, 116  
stability, 105  
sterilization, 115  
turbidostat, 103  
washout, 103
- Bioreactors for enzymatic reactions, 77     batch, 85  
          combinations, 92
- comparison of types, 91  
CSTR, 85     approach to steady state, 88  
          volume, 87  
dilution rate, 86  
fluidized-bed, 89  
hydraulic loading, 86  
plug-flow, 88  
residence time, 86  
steady state, 86  
stirred-tank, 85  
transport, 89
- Buffers, 6
- Carbon dioxide, solubility in media, 14  
Cell, death rate, 53, 62     flocs, 117  
          growth phases, 52  
          maintenance, 7, 56
- Cell growth inhibition, 63     competitive, 64  
          ethanol, 65  
          exponential, 66  
          Han-Levenspiel, 66  
          linear, 65  
          Lineweaver–Burk, 64  
          mixed, 64  
          photosynthesis, 74  
          pure non-competitive, 65  
          substrate, 63, 66  
          uncompetitive, 64
- Cell multiplication kinetics, 51     autotrophic, 74  
          Contois, 60  
          doubling time, 53  
          exerted growth, 60  
          first-order, 53  
          free growth, 60  
          Gompertz, 60  
          inhibition, 63

Cell multiplication kinetics (*Continued*)  
 limits, 75  
 logistic equation, 55, 59, 108  
 mass-transport limited, 69  
 mixed-cultures, 73  
 Monod kinetics, 54  
 Moser, 60  
 pellets, 70  
 pH effects, 62  
 piecewise-linear, 60  
 population limits, 55  
 Richards, 60  
 substrate limits, 54  
 temperature effects, 61  
 Tessier, 60  
 Verhulst–Pearl, 55, 59  
 Cellulose hydrolysis, 41  
*Clostridium puniceum*, 63  
 C-mole, 7  
 Commensalism in mixed cultures, 73  
 Competition, cell, 73  
 Contois equation, 60  
 Cooperativity, 26  
 Critical oxygen tension, 70  
 CSTR reactors, cell growth, 93, 94, 102  
   enzyme reactions, 85  
 Death phase, 52  
 Degree of reduction, 8  
 Diffusion, flux, 44  
   molecular, 44  
 Diffusivity, 44  
   liquids, 44  
*Dunaliella parva*, 60  
 Dilution rate, 86  
 Ecrinolysis in mixed cultures, 73  
 Effectiveness factor, 82  
   film, 83  
 Efficiency, cell growth, 9, 58  
   overall, for immobilized enzymes, 84  
   fermentation, 59  
 Energy, balances, 8  
   of combustion, 8  
 Enthalpy, of activation, 32  
 Entropy, 5  
 Enzymatic reactions, cellulose hydrolysis,  
   41  
   deactivation, 35

denaturation, 30, 33  
 immobilized enzymes, 78  
 inhibition, 35  
 mass-transfer with reaction, 80, 84  
 pH effects, 33  
 pressure effects, 31  
 temperature effects, 29, 31  
 Enzyme inhibition, 35  
   competitive, 36  
   mixed, 37  
   non-competitive, 37  
   pure non-competitive, 37  
   substrate, 28, 39  
   uncompetitive, 36  
 Enzyme kinetics, 19  
   bimolecular, 27  
   bimolecular, integral form, 28  
   inhibition, 35  
   Lineweaver–Burk equation, 37  
   single substrate, irreversible, 23  
   Michaelis–Menten general, 21  
   polymer hydrolysis, 41  
   rational equation, 26  
   reversible, 24  
   sigmoid, 27  
 Enzyme mechanisms, 28  
   ping-pong, 29  
   sequential, 28  
 Enzyme structure, 20  
   pyruvate dehydrogenase complex, 20  
 Enzymes, cells as enzymes, 77  
   immobilized, 77, 78  
   immobilized, effectiveness factor, 82  
   non-immobilized, 77  
 Equilibrium, carbon dioxide-water, 14  
   ethanol-water, 11  
   oxygen-water, 12  
   reaction, 16  
   temperature and pressure effects, 17  
   vapor-liquid, 11  
   water, 6  
   weak acid, 6  
 Exponential growth phase, 52  
 Eyring reaction rate equation, 31  
 Feeding in mixed cultures, 73  
 Fermentation, alcoholic, 58  
   efficiency, 58  
   process types, 57

- Fermenter, 2
- Fick's first law, 44
- Gaden fermentation classifications, 57
- Gibbs free energy, 5
  - of combustion, 8
- Gompertz equation, 60
- Grashof number, 47
- Haldane equation, 24, 63
  - Han–Levenspiel equations, 63, 66
  - substrate inhibition, 66
- Heat, dissipation, 9
  - of combustion, 8
  - production, 10
- Heifetz–Quinlan, 75, 114
- Henderson–Hasselbalch equation, 6
- Hill plot, 26
- Hydraulic loading, 86
- Inhibition, 35, 63
  - competitive, 36, 64
  - mixed, 37, 64
  - non-competitive, 38
  - pure non-competitive, 38, 65
  - reversibility, 35
  - substrate, 28, 39, 63, 66
  - time-dependent, 35
  - uncompetitive, 37, 64
- Ionization, 6
  - water, 6
- Lag phase, 52
- Length, characteristic, 79
- Lineweaver–Burk equation, 37, 64
- Log phase, 52
- Logistic equation, 55, 59, 108
- Maintenance coefficients, 56
  - temperature dependence, 56
- Mass transfer, 43, 69
  - Fick's first law, 44
  - gas-liquid, 45
  - immobilized enzyme systems, 78
  - liquid, 44
  - liquid-liquid, 49
  - membranes, 48
  - oxygen, 46, 69, 117
- solid-liquid, 50
- tubes, 48
- Michaelis–Menten equation, 21
  - general form, 22
  - integral form, 22
- Mixed cultures, 73
- Mixing specific power, 118
- Mixing time, 118
- Monod equation, 41, 54
- Moser equation, 60
- Mutualism in mixed cultures, 73
- Mycoplasma, 74
- Oxygen, critical oxygen tension, 70
  - mass transfer, 46, 70
  - solubility in media, 12
- Parasitism, 73
- Péclet number, 47
- Pellets, mycelial, 70
- pH, 6
  - effects on cell growth, 62
  - effects on enzymatic reactions, 33
- Photosynthesis, 74, 114
- Pirt equation, 71
- pK<sub>a</sub>, 6
- Plasmids, 73
- Plug-flow reactors, cell growth, 93, 107
  - enzymatic reactions, 88
- Polymer hydrolysis, 41
- Population doubling time, 53
- Power, compressor, 10
  - mixing, 118
- Predation in mixed cultures, 73
- Protocooperation in mixed cultures, 73
- Radius, equivalent, 79
- Reactions, dissimilation, 57
- Thermodynamics, 5
- Thiele modulus, 82
- Verhulst–Pearl equation, 55, 59
- Viruses, 74
- Weisz modulus, 83
- Wilke–Chang equation, 44
- Yield factors, 55, 58