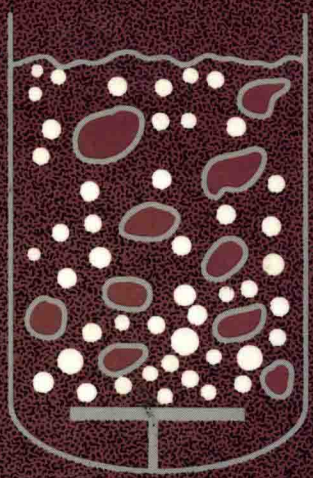
Bioreactor Design Fundamentals

Norton G. McDuffie



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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
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July 1991

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Contents

Preface ix

1 Introduction 1

2 Thermodynamics and Stoichiometry for Bioreactor Design 5

Thermodynamics 5
- Standard states 5
- pH and buffers 6
- Stoichiometry 7
- Energy balances 8
- Phase equilibrium relationships 11
- Reaction equilibrium 16

3 Enzyme Kinetics 19

Mathematical models for enzyme-catalyzed reactions 19
Effect of temperature on enzymatic reactions 29
Effect of pH on enzyme-catalyzed reactions 33
Enzyme inhibition 35
- Types of inhibitors 35
- Reversibility of inhibition 35
- Time-dependent deactivation 35
- Equilibrium inhibition models for Michaelis–Menten kinetics 36

Kinetics of polymer hydrolysis 41

4 Mass-Transfer Fundamentals 43

Mass-transfer limitations 43
Intraphase mass transfer 43
Gas–liquid mass transfer 45
Mass transfer through membranes 48
Mass transfer through porous structures 49
Interphase mass transfer at liquid–liquid interfaces 49
Mass transfer at solid–liquid interfaces 50
General mass-transfer considerations 50

5 Kinetics of Cellular Multiplication **51**

Nature of cell growth and multiplication 51
Monod kinetics 54
Stoichiometry for substrate and product relationships 55
Alternative mathematical models 59
Temperature effects on cell processes 61
Effects of other environmental factors 62
Cell growth inhibition 63
Mass-transport and physical restraint limitations 69
Mixed cultures 73
Autotrophic growth 74
Rate limit determinations 75

6 Enzyme Reactors **77**

Overview 77
Nonimmobilized enzyme systems 77
Immobilized enzyme systems 78
Stirred-tank bioreactors 85
Plug-flow bioreactors 88
Comparison of plug-flow and CSTR reactors 90
Reactor combinations 92

7 Cell Culture Bioreactors **93**

Types of biological growth reactors 93
CSTR biological growth systems 94
The batch stirred-tank culture system 96
Fed-batch cultures 100
Single continuous-flow CSTR without recycle 102
Graphic analysis of single and multiple CSTRs 104
Plug-flow reactors for cell growth 107
Recycle operations 111
Continuous culture of photosynthetic organisms 114
Sterilization of culture media 115
Scale-up of cell-culture bioreactors 116

List of Symbols 121

References 129

Index 135

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

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-

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

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

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

$$\text{pH} = pK_a + \log_{10} \frac{[A^-]}{[HA]}, \tag{2.2}$$

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

$$HA = H^+ + A^-. \tag{2.3}$$

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_2–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 ($C_6H_{12}O_6$) is the unit CH_2O. 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 $CH_{1.8}O_{0.5}N_{0.2}$ (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 $CH_{1.8}O_{0.5}N_{0.2}$ 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_3 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, γ_i, for this case is

$$\gamma_i = 4 + a_i - 2b_i - 3c_i, \tag{2.4}$$

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, $\Delta h^\circ_{c_i}$, and the standard-state Gibbs free energy of combustion, $\Delta g^\circ_{c_i}$, for a C-mole of component I are as follows:

$$\Delta h^\circ_{c_i} = 115\ \gamma_i \pm 18\ \text{kJ/C-mole}, \tag{2.5}$$

and

$$\Delta g^\circ_{c_i} = (94.4\gamma_i + 86.6) \pm 18\ \text{kJ/C-mole}. \tag{2.6}$$

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