Open-Ended Problems: A Future Chemical Engineering Education Approach. J. Patrick Abulencia and Louis Theodore.
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Biochemical Engineering

This chapter is concerned with biochemical engineering. As with all the chapters in Part II, there are sereval sections: Overview, several technical topics, illustrative open-ended problems, and open-ended problems. The purpose of the first section is to introduce the reader to the subject of biochemical engineering. As one might suppose, a comprehensive treatment is not provided although numerous references are included. The second section contains three open-ended problems; the authors' solutions (there may be other solutions) are also provided. The third (and final) section contains 40 problems; *no* solutions are provided here.

18.1 Overview

This overview section is concerned with—as can be noted from the chapter title—biochemical engineering. As one might suppose, it was not possible to address all topics directly or indirectly related to biochemical engineering. However, additional details may be obtained from either the

references provided at the end of this Overview and/or at the end of the chapter.

Note: Those readers already familiar with the details associated with biochemical engineering may choose to bypass this Overview.

Biochemical Engineering (BChE) is a relatively new discipline in the chemical engineering profession and, as one might suspect, it has come to mean different things to different people. Terms such as biomedical engineering, bioengineering, biotechnology, biological engineering, genetic engineering, etc., have been used interchangeable by many in the technical community. To date, standard definitions have not been created to distinguish between these terms. Consequently, the authors have lumped them all together using the term BChE for the sake of simplicity. What one may conclude from all the above is that BChE involves applying the concepts, knowledge, basic fundamentals, and approaches of virtually all engineering disciplines (not only chemical engineering) to solve specific health and health-care related problems in the biochemical field; the opportunities for interaction between chemical engineers and health-care professionals are therefore many and varied.

Because of the broad nature of this subject, this chapter can only serve as an introduction. The reader is referred to the three excellent references in the literature for an extensive comprehensive treatment of this discipline [1–3]. However, the bulk of the material in the chapter has been drawn from P. Vasudevan, "Biochemical Engineering," A Theodore Tutorial, Theodore Tutorials, East Williston, NY, a text originally published by the USEPA/APTI, RTP, NC, 1994 [4].

This subject has served as the title for numerous books. Condensing this subject matter into one chapter was a particularly difficult task. In the end, the authors decided to provide a superficial treatment that would introduce the reader to the subject. As noted, Vasudevan [4] provides an excellent and detailed review of key related topics.

Following this introductory section, the chapter consists of four sections:

1. Enzyme and Microbial Kinetics
2. Enzyme Reaction Mechanisms
3. Effectiveness Factors
4. Design Procedures

Additional information is available in the work of Vasudevan for the interested reader [4]. Also note that the notation adopted by Vasudevan is employed throughout this chapter.

18.2 Enzyme and Microbial Kinetics [4]

Enzyme and microbial kinetics involve the study of reaction rates and the variables that affect these rates. It is a topic that is critical for the analysis of enzyme and microbial reacting systems. The rate of a biochemical reaction can be described in many different ways. The most commonly used definition is similar to that employed for traditional reactors (see also Chapter 8). It involves the time change in the amount of one of the components participating in the reaction or in one of the products of the reaction; this rate is also based on some arbitrary factor related to the system size or geometry, such as volume, mass, and interfacial area. In the case of immobilized enzyme catalyzed reactions, it is common to express the rate per unit mass or volume of the catalyst.

The Michaelis-Menten rate equation is as follows

$$v = \frac{v_{max}[S]}{K_m + [S]} \tag{18.1}$$

where v = reaction rate = $-\frac{d[S]}{dt}$

v_{max} = constant defined as the maximum reaction rate

K_m Michaelis constant (dissociation constant)

[S]= substrate or reactan concentration

t= time

Note that when $K_m >> [S]$, the equation reduces to the following first order rate of reaction.

$$v = \frac{v_{max}}{K_m}[S] \tag{18.2}$$

For an enzyme reaction in which $[S] << K_m$

$$\ln\left(\frac{[S]}{[S]_o}\right) = \frac{v_{max}}{K_m} t \tag{18.4}$$

With respect to microbial cell growth, the rate equation can be written as:

$$\frac{d[X]}{dt} = \mu[X] \tag{18.4}$$

Integrating:

$$\ln\left(\frac{[X]}{[X]_o}\right) = \mu t \tag{18.5}$$

Where $[X]_o$ represents the initial cell concentration at t = 0.

Unlike chemical reactions both enzyme and microbial reactions are generally complex. The mechanism of enzyme catalyzed reactions is discussed in the next secion.

18.3 Enzyme Reaction Mechanisms

The rate of a chemical or biochemical reaction is similar to that defined earlier, i.e., the time rate or change in the quantity of a particular species participating in a reaction divided by a factor that characterizes the reacting system's geometry. The choice of this factor is also a matter of convenience. For homogeneous media, the factor is almost always the volume of the reacting system. For most fluid-solid reaction systems, the factor is often the mass of the solid. For example, in immobilized enzyme reactions, the factor is the mass of the immobilized or supported enzyme catalyst.

Consider the following scenario for a simple enzyme catalyzed reaction; the reaction scheme is as follows:

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrows}} ES \qquad ES \xrightarrow{k} E + P \tag{18.6}$$

where E = free enzyme
S = substrate or reactant
ES = primary enzyme-substrate complex
P = product
k = reaction rate constant

The decomposition of the primary complex ES to the free enzyme E and the product P is assumed to be the rate-determining (slow) step. The expression below is valid for both homogeneous (where the enzyme is used in the native or soluble form) and for immobilized enzyme reactions. The reaction rate v is given by:

$$v = -\frac{d[S]}{dt} = \frac{d[P]}{dt} \tag{18.7}$$

where [S] and [P] are once again the concentrations of substrate and product, respectively.

There are two approaches in deriving an expression for the reaction rate. In the *Michaelis-Menten approach* the first reaction in Equation (18.6) is assumed to be in equilibrium. The decomposition of the enzyme-substrate complex ES to form E and P is, as noted above, the rate-determining step. In the second approach, it is assumed that after an initial period, the rate of change of the concentration of the enzyme-substrate complex is essentially zero. Mathematically, this can be expressed as

$$\frac{d\left([ES]\right)}{dt} \approx 0 \tag{18.8}$$

This is known as the *quasi steady-state approximation*, and is valid for enzyme catalyzed reactions if the initial total enzyme concentration is much less than the initial substrate concentration, i.e., $[E]_o << [S]_o$.

The maximum reaction rate v_{max} is equal to $k[E]_o$. When $K_m = [S]$, $v = v_{max}/2$, one can derive an expression for the reaction rate. The total enzyme balance can be written as:

$$[E]_o = [E] + [ES] \tag{18.9}$$

where $[E]_o$ = the total enzyme concentration
[E] = concentration of free enzyme
[ES] = concentration of the enzyme-substrate complex

Since Michaelis-Menten kinetics is valid, the reaction between the free enzyme and substrate to from ES in Equation (18.6) may be assumed to be in equilibrium, so that

$$K_m = -\frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]} \tag{18.10}$$

where K_m is once again the Michaelis-Menten constant. The preceding equation can be combined with the total enzyme balance to provide a relationship between [ES] and the total enzyme concentration $[E]_o$.

$$[ES] = \frac{[E]_o[S]}{K_m + [S]} \tag{18.11}$$

The reaction rate v then equals:

$$v = k[ES] = \frac{k[E]_o[S]}{K_m + [S]} \tag{18.12}$$

In the quasi steady-state approximation, the constant K_m is known as the dissociation constant. Assuming quasi steady-state, the rate of disappearance of the enzyme-substrate complex, ES is:

$$\frac{d[ES]}{dt} = k_1[E][S] - k_1[ES] - k[ES] = 0 \tag{18.13}$$

Eliminating [E] by combining with the equation for the total enzyme balance, and solving for [ES],

$$[ES] = \frac{[E]_o[S]}{\left(\frac{k + k_{-1}}{k_1}\right) + [S]} \tag{18.14}$$

The reaction rate v then equals

$$v = k[ES] = \frac{k[E]_o[S]}{K_m + [S]} \tag{18.15}$$

which is identical to the expression obtained earlier; the only difference lies in the definition of K_m which is equal to $\frac{k+k_{-1}}{k_1}$ instead of $\frac{k_{-1}}{k_1}$ (equilibrium assumption).

18.4 Effectiveness Factor [4]

The effectiveness factor is defined as the ratio of the reaction rate in the presence of internal or pore diffusion to the reaction rate in the absence of pore diffusion. The value of the effectiveness factor is thus a measure of the extent of diffusion limitation. For isothermal reactions (generally true of most biochemical reactions), diffusional limitations are negligible when the effectiveness factor (η) is close to unity. If $\eta<1$, the reaction is diffusion limited.

The problem of pore diffusion is only limited to immobilized enzyme catalysts, and not enzyme catalyzed reactions in which the enzyme is used in the native or soluble form. The shape of the immobilized enzyme pellet may be spherical, cylindrical, or rectangular (as in a slab). If the reaction follows Michaelis-Menten kinetics as discussed above, a shell balance around a spherical enzyme pellet can be shown to result in the following second order differential equation:

$$D_e\left(\frac{d^2[S]}{dr^2}+\frac{2}{r}\frac{d[S]}{dr}\right)=\frac{v_{max}[S]}{K_m+[S]} \tag{18.16}$$

where D_e = the effective diffusivity, cm^2/s

The boundary conditions (BC) are:

1. $[S]=[S]_o$ at $r=R$ where R is the radius of the spherical catalyst pellet and $[S]_o$ is the substrate concentration in the bulk liquids, and
2. $D[S]/dr=0$ at $r=0$ (due to the symmetry of the pellet, the concentration gradient is zero at the center)

The preceding differential equation can be solved analytically or numerically to determine the concentration profile inside the pellet.

From the definition of the effectiveness factor, the actual or observed reaction rate, v_s (in the presence of pore diffusion), is equal to:

$$v_s = \eta v = \eta\left[\frac{v_{max}[S]_o}{K_m + [S]_o}\right] \tag{18.17}$$

When the substrate concentration is low, i.e., when $[S] << K_m$, the reaction rate becomes first order. In this case, the preceding differential equation can be solved analytically to obtain the concentration profile inside the catalyst pellet. By defining the following dimensionless parameters, the differential equation can be written in a dimensionless form as follows:

$$\bar{S} = \frac{[S]}{[S]_o},\ \bar{r} = \frac{r}{R} \tag{18.18}$$

and

$$\left(\frac{d^2S}{d\bar{r}^2} + \frac{2}{\bar{r}}\frac{d\bar{S}}{d\bar{r}}\right) = 9\phi^2\bar{S} \tag{18.19}$$

where

$$\phi = \frac{R}{3}\sqrt{\frac{v_{max}/K_m}{D_e}} = \text{the Thiele modulus} \tag{18.20}$$

Once the concentration profile is known, the effectiveness factor can be expressed as a function of the Thiele modulus by the following relationship:

$$\eta = \frac{1}{\phi}\left[\frac{1}{\tanh 3\phi} - \frac{1}{3\phi}\right] \tag{18.21}$$

The observed reaction rate can then be determined from Equation (18.17).

For a rectangular catalyst slab or a rectangular membrane in which both sides are exposed to the substrate, the effectiveness factor is related to the Thiele modulus as follows:

$$\eta = \frac{\tanh\phi}{\phi} \tag{18.22}$$

where

$$\phi = \frac{L}{2}\sqrt{\frac{v_{max} / K_m}{D_e}} \tag{18.23}$$

and L is the thickness of the membrane or catalyst slab.

18.5 Design Procedures

There are a host of design topics associated with biochemical reactors [5]. Discussing these in any detail is also beyond the scope of this book. Topics of interest to the practicing chemical engineer are primarily concerned with sterilization. Four specific areas include:

1. Design of a batch sterilization unit
2. Design of a continuous sterilization unit
3. Design of an air sterilizer
4. Scale-up of a fermentation unit

Each of these briefly receives qualitative treatment below with Vasudevan [4] providing quantitative details and analyses plus illustrative examples.

18.5.1 Design of a Batch Sterilization Unit

Sterilization is the process of inactivation or removal of viable organisms. Sterilization can be accomplished by the steaming of equipment and/or medium, plus the additives; it is an important operation in the fermentation industry. The main objective of media sterilization is to kill all living organisms present before inoculation and to eliminate any possible competition or interference with the growth and metabolism of the desired organism. This objective should be accomplished with minimal damage to the media ingredients.

One of the drawbacks of batch sterilization is that Del factors [4], which is a measure of the extent of sterilization, are scale dependent. Large fermenters require longer heating and cooling times. The consequences of longer times can be severe if the medium is thermolabile since the destruction due to heat is dependent on the value of the thermal rate constant. A better alternative for heat sensitive material is to use continuous sterilization, a topic discussed in the next subsection.

18.5.2 Design of a Continuous Sterilization Unit

As noted above, the consequences of long heat-up and cool-down times in batch sterilization can be severe if the medium components are heat-sensitive. Since the destruction due to heat is dependent on the value of the *thermal death constant* [4] it can be shown that the spore B. *stearothermophilus* is only significantly inactivated above 110°C due to its high activation energy of 67.7 kcal/mol. On the other hand, many organic nutrients, which follow the Arrhenius relationship for thermal degradation, have a much lower activation energy of approximately 25-30 kcal/gmol. This analysis implies that longer exposure to lower sterilization temperatures, due to slow heat-up or cool-down, can cause more damager to nutrients. The best alternative for heat sensitive materials is to use continuous sterilization.

In continuous sterilization, the raw medium is mixed with water, and then continuously pumped through the sterilizer to a sterilized fermenter. In the sterilizer, the media is instantaneously heated by either direct or indirect contact with steam [6], and held at a very high temperature (about 140°C) for a relatively short time. The residence time or holding time of the medium is fixed by adjusting the flow rate and length of the insulated holding pipe. The hot steam from the sterilizer is rapidly cooled by a heat exchanger (with or without heat recovery) and/or by flash cooling before it enters the fermenter.

The design of continuous sterilizers must also allow some flexibility in operating conditions to adapt the system to a different medium. The system must incorporate automatic recycle to recirculate the medium if the temperature falls below the design value. The design should include:

1. The ability to fill the fermenter within 2-3 hours (this might be considered down time)
2. The recovery of 60-70% of the heat
3. Plug flow in the holding section
4. The option of either direct of indirect heating

The continuous sterilization process has several advantages.

1. The temperature profile of the medium is almost one of instant heating and cooling, allowing an easy estimate of the Del factor required [4].
2. Scale-up is very simple since the medium is exposed to high temperatures for very short times, thereby minimizing nutrient degradation.

3. The energy requirements of the sterilization process can be dramatically reduced by using the incoming raw medium to cool the hot sterile medium.

The difficulties with continuous sterilizers are typically due to exchanger fouling and control instability. In general, any medium containing starches requires special attention.

18.5.3 Design of an Air Sterilizer [4]

In aerobic fermentations, it is necessary to sterilize air. Since the volume of air required in aerobic fermentations is usually large, conventional techniques of heat sterilization are uneconomical. Effective and viable alternatives include the use of membranes or fibrous filters. An important consideration of the filter medium is that it should not be wetted since this can lead to deposition contamination. Materials such as glass fibers are often used to avoid this problem.

The mechanisms by which particles suspended in a flowing stream of air are removed include *impaction, diffusion, and interception* [7]. Impaction occurs when particles in the air collide with the fibrous filter due to their higher momentum as compare to air. Smaller particles, on the other hand, travel towards the fiber as a result of molecular diffusion caused by Brownian motion; particles less than 1μm are collected by this mechanism [7,8]. Particles that are not small or heavy but large in size may be intercepted by the fiber. The efficiency of air filtration is therefore a combination of the three mechanisms.

The mathematical equations for designing air sterilizers may be developed by considering the effect of each of the above mechanisms separately, and then developing a combined expression. In the case of collision or impaction, the efficiency of the process is a function of the Reynolds (Re) and Stokes (St) numbers with the Reynolds number being based on the diameter of the filter or collection device.

The effect of the air velocity on efficiency is considerable. Removal of particles by collision and interception is enhanced as the air velocity is increased, whereas the efficiency of particulate removal by diffusion is lowered because of the residence time. This is of course dependent on the particle size [7]. In general, it should be remembered that most equations for the removal efficiency are empirical.

18.5.4 Scale-Up of a Fermentation Unit

Scale-up is a fundamental problem in the fermentation industry because of the need to perform microbial operations in different size equipment. The

scale-up problem arises from the difference in transport phenomena when the scale and geometry of the equipment is changed. Describing transport and kinetic phenomena in fermenters requires knowledge of both the kinetics and flow patterns. Since the balances are very complex and the flow patterns are largely unknown, the use of fundamental principles for design is limited. Instead, various empirical procedures are usually employed.

The first step in scaling up a fermentation process is to use the production requirements to determine the size and number of fermenters that will be required. In sizing a new unit, or evaluating an existing fermenter for a biological process, it is necessary to establish the desired product rate R on an annual or daily basis. Then the productivity of the individual fermenter – defined as the weight of product that the fermenter can produce per unit volume per unit time –may be determined.

In addition to the fermentation phase, the overall productivity must account for the time spent in activities such as cleaning, filling and sterilizing; this is normally referred to as *down-time*. This is important if the process is to be operated batch-wise. The total installed fermenter capacity, V, required can then be calculated by dividing the desired product rate by the productivity. The number of fermenters can then be easily calculated. The next step is to size the number of inoculum stages. Fermenter pre-cultures must be made in order to have sufficient inoculum for a large fermenter. The production fermenter volume and the optimal inoculum levels will determine how many pre-culture stages are necessary. Typical inoculum concentrations are:

Bacteria (0.1-3%)
Actinomycetes (5-10%)
Fungi (5-10%)

A detailed and expanded treatment of this topic is available in the following two references.

1. P. Vasudevan, *Biochemical Engineering*, A Theodore Tutorial, Theodore Tutorials, East Williston, NY, a text originally published by the USEPA/APTI, RTP, NC, 1994 [1].
2. Adapted from, L. Theodore, *Chemical Engineering: The Essential Reference*, McGraw-Hill, New York City, NY, 2014 [1].

18.6 Illustrative Open-Ended Problems

This and the last section provide open-ended problems. However, solutions *are* provided for the three problems in this section in order for the reader

to hopefully obtain a better understanding of these problems which differ from the traditional problems/illustrative examples. The first problem is relatively straightforward while the third (and last problem) is somewhat more difficult and/or complex. Note that solutions are not provided for the 40 open-ended problems in the next section.

Problem 1: The kinetics of mixed cell cultures are very important in the biological treatment of wastewater. Mixed cell populations exist in a number of natural environments, and it is very important to understand how these cells interact with one another. Define and discuss the following terms:

1. Neutralism
2. Mutalism
3. Symbiosis
4. Methanobacillus omelianskii
5. Competition
6. Commensalism
7. Ammensalism

Also discuss the predator-prey problem.

Solution: *Neutralism* refers to mixed growth of two microbial populations in which the growth rate of either microorganism is not affected by the presence of the other. On the other hand, *mutualism* refers to a case where both populations grow faster together. This may be due to the exchange of growth factors or nutrients. If the partnership between the two species is necessary for the survival of either species, then the mutualistic relationship is referred to as *symbiosis*. In anaerobic sludges, *methanobacillus amelianskii*, has been found to be a mixture of two species. The first species of the bacterium converts ethanol to hydrogen and acetate, but is inhibited by the product hydrogen. The second species consumes hydrogen and produces methane. *Competition* occurs when each species exerts a negative influence on the other. If the second microbial species alone enjoys the benefits of interacting with the first one, this relationship is referred to as *commensalism*. If the growth of the second species is affected by the first population, the process is known as *amensalism*.

Multiple interacting species can give rise to complex behavior. For example, in predation and parasitism, one species benefits at the expense of the other. The consumption of bacteria by protozoa is a classic example

of a *predator-prey* relationship. The populations of predator and prey do not reach steady state values but oscillate for the protozoan-bacterium system. If the concentration of prey is high and the concentration of predator is low, the number of predators increases while the prey population simultaneously decreases. When the population of prey is sufficiently low, the predator population declines and the prey population starts to increase. This cycle will then repeat again and again.

Problem 2: Discuss why recombinant DNA has revolutionized the biotechnology industry.

Solution: This technique involves the production of a hybrid gene by joining pieces of DNA from different organisms *in vitro*, and then inserting this hybrid material into a host cell. As a result of developments in recombinant DNA (rDNA), there exists vast potential for directing cells to synthesize new products in the agriculture, chemical, environment, food and pharmaceutical industries. Some of the valuable products that have resulted from rDNA include insulin, interferon, human growth hormone and human serum albumin.

There are four basic steps common to genetic engineering. First, break up the DNA into short stretches or sequences. Second, join each bit into a suitable vector. Third, have the vector invade the host cells one at a time, and grow into large numbers. Finally, find the cells with the right transplanted genes in them.

In the first step, the DNA to be engineered is broken into small pieces by enzymes (known as restriction endonucleases) that recognize a particular sequence, and cut the DNA double helix only where such a sequence appears. The resultant shorter sequences of DNA often have "sticky ends". In the second step, the DNA is inserted into a vector, which is usually a bacterial plasmid (a circular double-stranded DNA molecule). The plasmid is cleaved by the same enzyme as used for the DNA to be transplanted, leaving sticky ends complementary to those of the DNA. Bits of DNA pair up and create a recombined plasmid. The third step involves the growth of bacteria carrying recombinant DNA. The plasmids are mixed with the host bacteria (such as *E. coli*), and the bacteria carrying recombinant plasmids are grown on the surface of a culture medium. Each bacteria divides until a colony of millions of bacteria are formed. A library of bacteria containing recombinant plasmids has, somewhere within it, the gene of interest. This can be identified and isolated.

Several types of instability may limit the productivity of recombinant DNA. First, a certain fraction of new cells may be born without plasmids. The plasmid-free cells may outgrow the plasmid-bearing cells. This situation

arises because the plasmid-bearing cells are at a disadvantage when competing with plasmid-free cells for essential nutrients. This is known as segregational instability. On the other hand structural instability results in the inability of cells to synthesize the active product. Many methods are being pursued to genetically eliminate plasmid instability. For example, the application of *selective pressure*, such as the addition of an antibiotic supplement to the medium will kill plasmid-free cells since plasmids contain a marker for antibiotic resistance. Cells containing the plasmid will not be affected as a result. Growth-rate dominated instability or growth-rate differential occurs as a result of the redirection of cellular (catabolic and anabolic) activity in the recombinant cells when they synthesize the desired product. Consequently, plasmid-bearing cells do not have full use of their own resources and grow more slowly than those which are plasmid-free. The plasmid-free cells born by plasmid segregation are thus able to rapidly overtake the population of plasmid-bearing cells. To alleviate this problem, a two stage fermentation process can be used in which the first stage is optimized to produce viable plasmid containing cells, and the second stage is used to produce the target protein by turning on an inducing promoter.

Problem 3: Bovine liver catalase is used to decompose hydrogen peroxide to water and oxygen. The concentration of hydrogen peroxide is given in Table 18.1 as a function of time for a reaction mixture at 25°C with a pH of 7.0.

Table 18.1 Time-Concentration Data for Problem 3.

Time, min	0	10	20	50	100
H_2O_2, molar concentration	0.02	0.018	0.016	0.011	0.005

Determine the Michaelis-Menten parameters by any suitable means. If the total concentration of the enzyme changes, what will the substrate concentration be after various periods of time?

Comment: Refer to the work of P.T. Vasudevan [4] for additional details.
Solution: The rate (v) equation for the quasi steady-state approximation is given by:

$$v = -\frac{d[S]}{dt} = \frac{v_{max}[S]}{K_m + [S]} \tag{18.1}$$

This equation can be integrated between the limits $[S]_o$ (initial substrate concentration and $[S]$ (substrate concentration at time t):

$$\int_{[S]_o}^{[S]} \frac{K_m + [S]}{[S]} = -\int_0^t v_{max} dt \tag{18.24}$$

One may linearize the integrated form of the equation. The resulting equation is implicit in the substrate (reactant) concentration. A numerical linear regression analysis provide estimates of the two parameters [10]. Or, a graphical technique can be used and the parameters estimated from the slope and intercept.

$$\frac{[S]_o - [S]}{\ln\frac{[S]_o}{[S]}} = \frac{v_{max} t}{\ln\frac{[S]_o}{[S]}} - K_m \tag{18.25}$$

A plot of $\frac{[S]_o - [S]}{\ln\frac{[S]_o}{[S]}}$ versus $\frac{t}{\ln\frac{[S]_o}{[S]}}$ should be linear with an intercept = $-K_m$ and a slope = v_{max}. Employing a numerical linear regression analysis, v_{max} = 3.605 x 10^{-4} mol/L·min and K_m = 0.015 M.

Assume the enzyme concentrations is now doubled so that v_{max} will be twice the value estimated above. The reaction rate constant k is an intrinsic parameter that does not change. Thus, $v_{max} = 2 \times 3.605 \times 10^{-4} = 7.21 \times 10^{-4}$ mol/Lmin.

Substituting into the integrated equation above and calculate the substrate concentration for the conditions specified. This requires an iterative procedure. The substrate concentration is given by

$$[S] = [S]_o - v_{max} t + K_m \ln\frac{[S]_o}{[S]} \tag{18.25}$$

Substituting t = 30 minutes, $[S]_o$ = 0.02 M, v_{max} 7.21 x 10^{-4} mol/L·min and K_m = 0.015 M into the above equation and solving iteratively leads to

$$[S] = 0.0095 \text{ M}$$

The above calculation can be repeated for other periods of time and other total enzyme concentrations.

Finally, the reader should note that integral analysis of reaction data in enzyme catalyzed reactions does not yield very accurate values of the reaction parameters. It is important to measure initial reaction rates since the quasi steady-state approximation is not valid as $[S]$ approaches $[E]_0$.

18.7 Open-Ended Problems

This last section of the chapter contains open-ended problems as they relate to biochemical engineering. No detailed and/or specific solution is provided; that task is left to the reader, noting that each problem has either a unique solution or a number of solutions or (in some cases) no solution at all. These are characteristics of open-ended problems described earlier.

There are comments associated with some, but not all, of the problems. The comments are included to assist the reader while attempting to solve the problems. However, it is recommended that the solution to each problem should initially be attempted *without* the assistance of the comments.

There are 40 open-ended problems in this section. As stated above, if difficulty is encountered in solving any particular problem, the reader should next refer to the comment, if any is provided with the problem. The reader should also note that the more difficult problems are generally located at or near the end of the section.

1. Describe the early history associated with biochemical engineering.
2. Discuss the recent advances in biochemical engineering.
3. Select a refereed, published article on biochemical engineering from the literature and provide a review.
4. Provide some normal everyday domestic applications involving the general topic of biochemical engineering.
5. Develop an original problem on biochemical engineering that would be suitable as an illustrative example in a book.
6. Prepare a list of the various technical books which have been written on biochemical engineering. Select the three best and justify your answer. Also select the three weakest books and, once again, justify your answer.
7. Discuss both enzyme and microbial kinetics.
8. Discuss the general subject of enzyme reaction mechanisms in layman terms.

9. Provide the equations employing traditional chemical engineering notation for the Michaelis-Menten approach to describing expressions for the rate of reaction.
10. Provide the equations employing traditional chemical engineering notation for describing expressions for the quasi steady-state approach.
11. Define and discuss the effectiveness factor.
12. Provide a layman's definition of the Thiele modulus.
13. Provide a layman's definition of the biological oxygen demand (BOD).
14. Provide a layman's definition of the chemical oxygen demand (COD).
15. Provide a layman's definition of the total organic carbon (TOC).
16. Describe the various biomedical engineering opportunities that exist for chemical engineers.
    Comment: Refer to the work of Abulencia and Theodore [11] for additional details.
17. Define and discuss the power number as it applies in the design of agitated tanks.
18. Three dimensionless numbers involved in particulate behavior are the Peclet, Stokes, and Schmidt numbers [7]. Define these numbers and discuss the relationship between the three.
19. Describe the Cunningham Correction Factor (CCF) [7]. Attempt to improve on this equation.
    Comment: Refer to the literature [7,8] for additional details.
20. Describe the batch sterilization process.
21. Discuss the various types of heat transfer mechanisms employed in batch sterilization.
22. Define and describe both competitive inhibition and substrate inhibition.
23. Discuss the general subject of immobilized enzymes and enzyme deactivation. Are there any major differences?
24. Discuss metabolism, catabolism, and anabolism. What are the differences?
25. Describe Monod growth kinetics in layman terms.
26. Discuss the role membrane separators [12] plays in biochemical engineering processes.
27. In terms of biochemical concepts, provide a brief definition and description of

- bacteria
- viruses
- cells
- fungi
- mutation

6. genetic concepts

28. Describe the different phases of bacteria cell growth.
29. Provide a layman's definition of chemostats.
30. Discuss the difference between biochemical and biomedical engineering.
31. Provide fluid flow analogies with the following biomedical terms:
    - Blood
    - Blood vessels
    - Heart
    - Plasma cell flow

    Comment: Refer to the literature [11] for additional details.
32. List and briefly describe the various classes of biochemical reactors.

    Comment: Refer to the literature for additional details [5].
33. Describe trickling biological filters.
34. Instead of a Lineweaver-Burke plot, can you think of any other way of linearizing the describing equation and deducing the nature of inhibition? Can the nature of inhibition and the inhibition parameter be determined from a Dixon plot? Discuss the significance of a high inhibition constant.

    Comment: Refer to the literature [4]
35. Describe fluidized bed bioreactors and discuss the differences with the traditional fluid bed reactors.
36. Describe how the immobilization of cells on an inert support is carried out.
37. Explain why scale-up is a fundamental problem in the fermentation industry.
38. Consider the growth of an organism that follows Monod growth kinetics in a batch reactor. If the yield $Y_{x/s} = 0.5$, $\mu_{max} = 0.5\ h^{-1}$, $K_s = 0.004$ g/L, the initial cell concentration $[X]_o = 1.0$ g/L, and the intial substrate concentration $[S]_o = 20$ g/L, provide equations on how ln[X], [X], [S], d[X]/dt vary with respect to time.

    Comment: Refer to the literature [4] for additional details.

39. A simple batch fermentation gave the results provided in Table 18.2.
    Calculate the following:
    - Yield
    - Maximum growth rate
    - Doubling time
    - Lag time

    Specific growth rate of various times.
    Provide a general analysis of the results.
    Comment: Refer to the work of Vasudevan [4] for additional details.
40. Data for the enzymatic breakdown of a phosphate chemical is provided in Table 18.3.

Table 18.2 Time-Concentration Data for Problem 39.

Time, min	$[X]$, g/L	$[s]$, g/L
0	0.19	9.2
120	0.21	9.2
240	0.31	9.1
480	1.0	8.0
600	1.8	6.8
720	3.2	4.6
840	5.6	0.9
960	6.2	0.08
1080	6.3	0

Table 18.3 Rate-Concentration Data for Problem 40.

Substrate concentration, μmol/L	Initial rate, μmol/L·min at initial inhibitor concentration, μmol/L	
	[i] = 0.0	[i] = 146
6.7	0.300	0.1075
3.5	0.238	0.0800
1.7	0.160	0.0562

What can one deduce about the nature of the inhibition?
Comment: Refer to the literature [4] for additional details.

References

1. J. Enderle, S. Blanchard, and J. Bronzing, *Introduction to Biomedical Engineering*, 2nd ed, Elseview/Academic Press, New York City, NY, 2000.
2. J. Bronzing (editor), *Biomedical Engineering Fundamentals*, 3rd ed, CRC/Taylor & Francis Group, Boca Raton, FL, 2000.
3. S. Vogel, *Life in Moving Fluids*, 2nd ed, Princeton University Press, Princeton, NJ, 1994.
4. P. Vasudevan, *Biochemical Engineering*, A Theodore Tutorial, Theodore Tutorials, East Williston, originally published by the USEPA/APTI, RTP, NC, 1994.
5. Adapted from: L. Theodore, *Chemical Reactor Design and Analysis for the Practicing Engineer*, John Wiley & Sons, Hoboken, NJ, 2012.
6. L. Theodore, *Heat Transfer for the Practicing Engineer*, John Wiley & Sons, Hoboken, NJ, 2011.
7. L. Theodore, *Air Pollution Control Equipment Calculations*, John Wiley & Sons, Hoboken, NJ, 2008.
8. C.E. Cunningham, *Proc. Roy. Soc. London*, Ser. A, 83, 357, 1910.
9. L. Theodore, *Chemical Engineering: The Essential Reference*, McGraw-Hill, New York City, NY, 2014.
10. S. shaefer and L. Theodore, *Probability and Statistics Applications in Environmental Science,* CRC Press/ Taylor & Francis Group, Boca Raton, FL, 2007.
11. P. Abulencia and L. Theodore, *Fluid Flow for the Practicing Chemical Engineer*, John Wiley & Sons, Hoboken, NJ, 2009.
12. L. Theodore and F. Ricci, *Mass Transfer Operations for the Practicing Engineer*, John Wiley & Sons, Hoboken, NJ, 2010.