Chapter 27

Enzyme Fermentation

The term "fermentation" can be used in its original strict meaning (to produce alcohol from sugar—nothing else) or it can be used more or less broadly. We will use the modern broad definition:

> *From the simplest to the most complex, biological processes may be classed as fermentations, elementary physiological processes, and the action of living entities. Further, fermentations can be divided into two broad groups: those promoted and catalyzed by microorganisms or microbes (yeasts, bacteria, algae, molds, protozoa) and those promoted by enzymes (chemicals produced by microorganisms). In general, fermentations are reactions wherein a raw organic feed is converted into product by the action of microbes or by the action of enzymes.*

This whole classification is shown in Fig. 27.1.

Enzyme fermentations can be represented by

$$\text{(organic feed, A)} \xrightarrow[\text{acting as catalyst}]{\text{enzyme E}} \text{(product chemical, R)} \qquad \textbf{(1)}$$

Microbial fermentations can be represented by

$$\text{(organic feed, A)} \xrightarrow[\text{which acts as catalyst}]{\text{microbe C}} \text{(product, R)} + \text{(more cells, C)} \qquad \textbf{(2)}$$

The key distinction between these two types of fermentation is that in enzyme fermentation the catalytic agent, the enzyme, does not reproduce itself, but acts as an ordinary chemical, while in microbial fermentation the catalytic agent, the

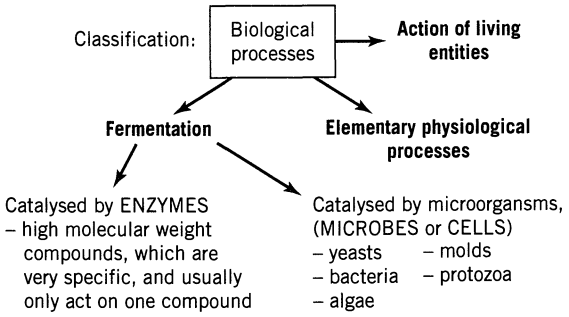


Figure 27.1 Classification of biological processes.

cell or microbe, reproduces itself. Within the cells it is the enzyme which catalyses the reaction, just as in enzyme fermentation; however, in reproducing itself the cell manufactures its own enzyme.

In this chapter we introduce enzyme fermentations, in the following chapters we take up microbial fermentations.

27.1 MICHAELIS–MENTEN KINETICS (M–M KINETICS)

In a sympathetic environment, with just the right enzyme for catalyst, organic A will react to produce R. Observations show the behavior of Fig. 27.2.

A simple expression which accounts for this behavior is

$$-r_A = r_R = k \frac{C_{E0}\, C_A}{C_M + C_A} \qquad (3)$$

(C_{E0}: total enzyme; C_M: a constant, called the Michaelis constant)

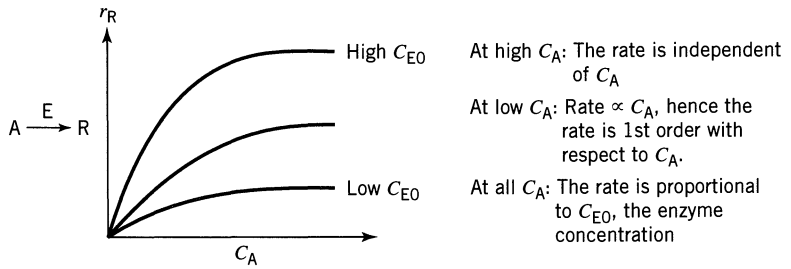


Figure 27.2 Typical rate-concentration curves for enzyme catalyzed reactions.

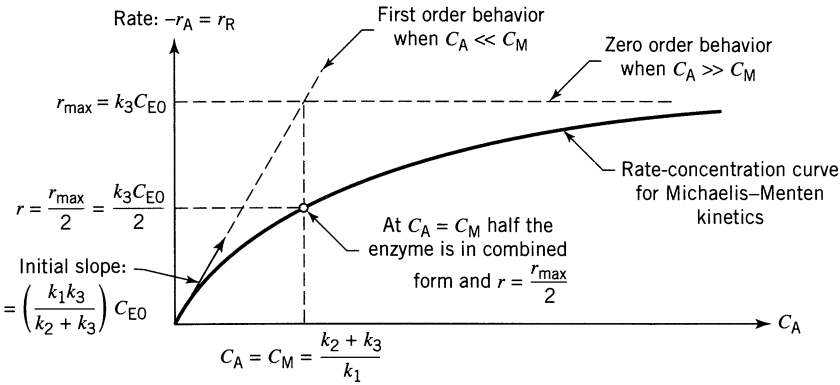


Figure 27.3 Special features of the M–M equation, Eq. 3.

In searching for the simplest mechanism to explain these observations and this rate form, Michaelis and Menten (1913) came up with the two-step elementary reaction mechanism

$$A + E \underset{2}{\overset{1}{\rightleftharpoons}} X \xrightarrow{3} R + E \quad \text{... and ...} \quad C_{E0} = C_E + C_X \tag{4}$$

intermediate; free enzyme; total enzyme; enzyme attached to reactant

Example 2.2 develops and explains the relationship between the above mechanism and rate equation. Let us look at some of the special features of the Michaelis–Menten equation.

- when $C_A = C_M$ half the enzyme is in free form, the other half combined.
- when $C_A \gg C_M$ most of the enzyme is tied up as complex X.
- when $C_A \ll C_M$ most of the enzyme is in free form.

Graphically we show this equation in Fig. 27.3.

Next let us see how to evaluate the two rate constants of this important enzyme fermentation equation.

Batch or Plug Flow Fermentor

For this system, integration of the M–M equation gives [see Eq. 3.57 or see Michaelis and Menten (1913)]

$$\underbrace{C_M \ln \frac{C_{A0}}{C_A}}_{\text{first-order term}} + \underbrace{(C_{A0} - C_A)}_{\text{zero-order term}} = k_3 C_{E0} t \tag{5}$$

This concentration-time behavior is shown in Fig. 27.4.

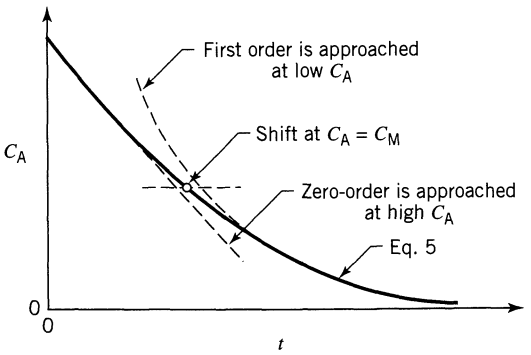


Figure 27.4 Concentration-time behavior of the M–M equation.

Unfortunately this equation cannot be plotted directly to find the values of the constants k_3 and C_M. However, by manipulation we find the following form which can be plotted, as shown in Fig. 27.5, to give the rate constants

$$\frac{C_{A0} - C_A}{\ln \dfrac{C_{A0}}{C_A}} = -C_M + k_3 C_{E0} \cdot \frac{t}{\ln \dfrac{C_{A0}}{C_A}} \tag{6}$$

Mixed Flow Fermentor

Inserting the M–M equation into the mixed flow performance expression gives

$$\tau = \frac{C_{A0} - C_A}{-r_A} = \frac{(C_{A0} - C_A)(C_M + C_A)}{k_3 C_{E0} C_A} \quad \ldots \text{or}$$

$$k_3 C_{E0} \tau = \frac{(C_{A0} - C_A)(C_M + C_A)}{C_A} \tag{7}$$

Unfortunately we cannot devise a plot of this equation to give k_3 and C_M. However, on rearrangement we find an equation form which does allow a direct evaluation of k_3 and C_M, or

$$C_A = -C_M + k_3 \left(\frac{C_{E0} C_A \tau}{C_{A0} - C_A} \right) \tag{8}$$

In graphical form this gives (see Fig. 27.6).

Alternative Methods for Evaluating k and C_M

Biologists and life scientists have developed a tradition of fitting and extracting the rate constants of the M–M equation with a multistep procedure, as follows

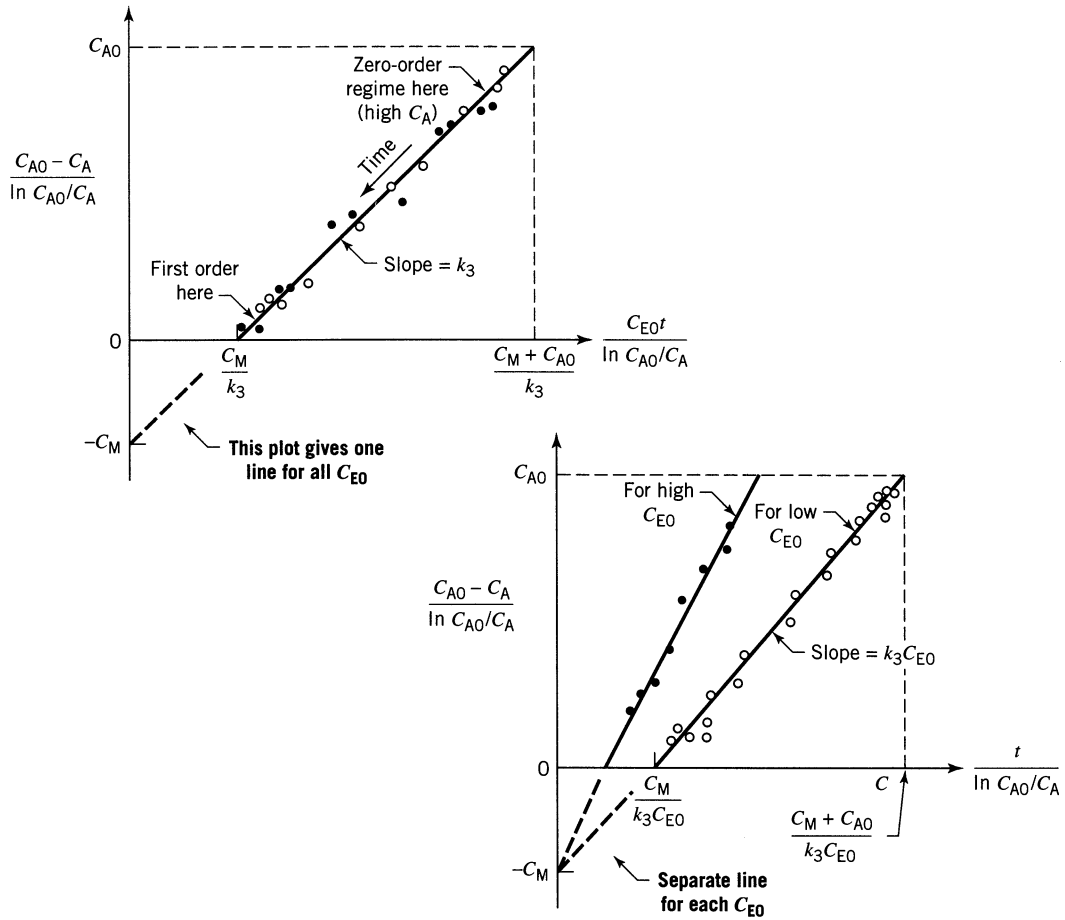


Figure 27.5 Either plot can be used to test and fit the M–M equation (Eq. 6) from batch reactor data.

- first measure C_{Aout} versus τ for data taken in any one of the three ideal reactors—batch, mixed, or plug flow.
- then evaluate $-r_A$ at various C_A, either directly from mixed flow data ($-r_A = (C_{A0} - C_{Aout})/\tau$) or by taking slopes for the plug flow or batch data, as shown in Chapter 3.
- plot C_A versus $(-r_A)$ in one of two ways

$(-r_A)$ versus $(-r_A)/C_A$ . . . the Eadie plot
$1/(-r_A)$ versus $1/C_A$ . . . the Lineweaver plot

- and from these plots extract C_M and k.

The CRE method which leads to Eq. 6 or Eq. 8 fits the measured C_A versus τ data from any of the three ideal reactor types, is direct, is less prone to fiddling, and is more reliable.

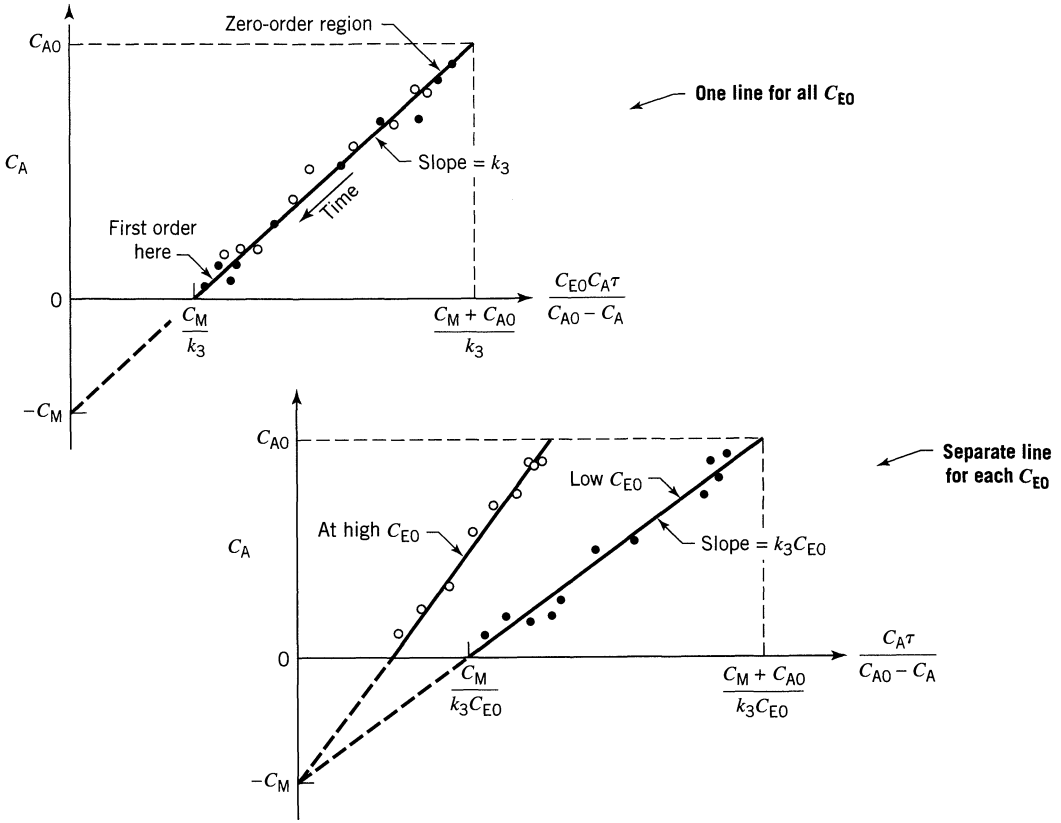


Figure 27.6 Either plot can be used to test and fit the M–M equation (Eq. 8) using data taken in a mixed-flow reactor.

27.2 INHIBITION BY A FOREIGN SUBSTANCE—COMPETITIVE AND NONCOMPETITIVE INHIBITION

When the presence of substance B causes the slowdown of the enzyme-substrate reaction of A to R, then B is called an *inhibitor.* We have various kinds of inhibitor action, the simplest models being called *competitive* and *noncompetitive.* We have competitive inhibition when A and B attack the same site on the enzyme. We have noncompetitive inhibition when B attacks a different site on the enzyme, but in doing so stops the action of A. In simple pictures this action is shown in Fig. 27.7.

Pharmacological significance: the study of enzymes and inhibition is one of the major methods in determining the action of existing drugs and in developing new drugs. This approach has changed the whole direction of pharmacological research in recent years. The main thrust today is

- to study the disease biochemically, then
- synthesize a chemical to block the action of a crucial enzyme.

Let us develop kinetic expressions for these two types of inhibition.

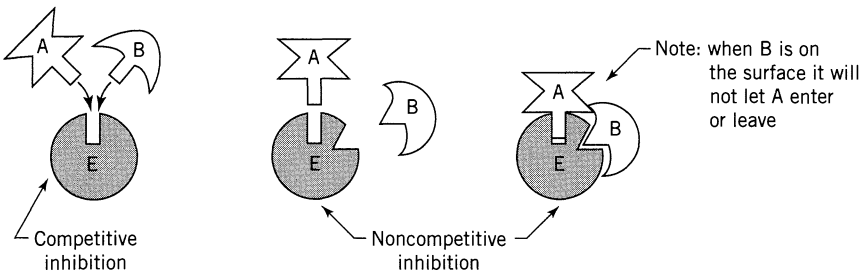


Figure 27.7 A simple representation of the action of two types of inhibitors.

Kinetics of Competitive Inhibition

With A and B competing for the same site on the enzyme we have the following mechanism:

$$A + E \underset{2}{\overset{1}{\rightleftarrows}} X \xrightarrow{3} R + E \tag{9}$$

$$B + E \underset{5}{\overset{4}{\rightleftarrows}} Y \tag{10}$$

note that B only attacks the free enzyme

With a procedure as shown in Chapter 2 (see Example 2.2) we end up with the following rate equation:

$$r_R = \frac{k_3 C_{E0} C_A}{C_M + C_A + N C_{B0} C_M} = \frac{k_3 C_{E0} C_A}{C_M(1 + N C_{B0}) + C_A}$$

$$\text{where} \quad \begin{cases} C_M = \dfrac{k_2 + k_3}{k_1}, \dfrac{\text{mol}}{\text{m}^3} \\ N = \dfrac{k_4}{k_5}, \dfrac{\text{m}^3}{\text{mol}} \end{cases} \tag{11}$$

Compared to systems without inhibition (Eq. 3) we see that all we need do here is replace C_M by $C_M(1 + NC_{B0})$.

Kinetics of Noncompetitive Inhibition

Here A attacks one site on the enzyme, B attacks a different site, but in doing so it stops the action of A. The mechanism of this action is represented by

$$A + E \underset{2}{\overset{1}{\rightleftarrows}} X \xrightarrow{3} R + E \tag{12}$$

$$B + E \underset{5}{\overset{4}{\rightleftarrows}} Y \tag{13}$$

$$B + X \underset{7}{\overset{6}{\rightleftarrows}} Z \tag{14}$$

Note that B attacks the enzyme irrespective of whether A is attached to it or not. The overall rate is then

$$r_R = \frac{k_3 C_{E0} C_A}{C_M + C_A + N C_{B0} C_M + L C_A C_{B0}}$$

$$= \frac{\dfrac{k_3}{(1 + L C_{B0})} \cdot C_{E0} C_A}{C_M \left(\dfrac{1 + N C_{B0}}{1 + L C_{B0}} \right) + C_A} \quad \text{... where} \quad \begin{cases} C_M = \dfrac{k_2 + k_3}{k_1} \\ N = \dfrac{k_4}{k_5} \\ L = \dfrac{k_6}{k_7} \end{cases} \tag{15}$$

Compared to enzyme reactions without inhibition we see that both k_3 and C_M are modified here. Thus, for noncompetitive inhibition,

- replace k_3 by $\dfrac{k_3}{1 + L C_{B0}}$
- replace C_M by $C_M \left(\dfrac{1 + N C_{B0}}{1 + L C_{B0}} \right)$

How to Tell between Competitive and Noncompetitive Inhibition from Experiment

With C versus t data obtained from either batch, mixed flow or plug flow runs make one of the recommended plots for inhibition-free systems (see Fig. 27.5 or Fig. 27.6). With inhibition these plots are modified as shown in Figs. 27.8 and 27.9.

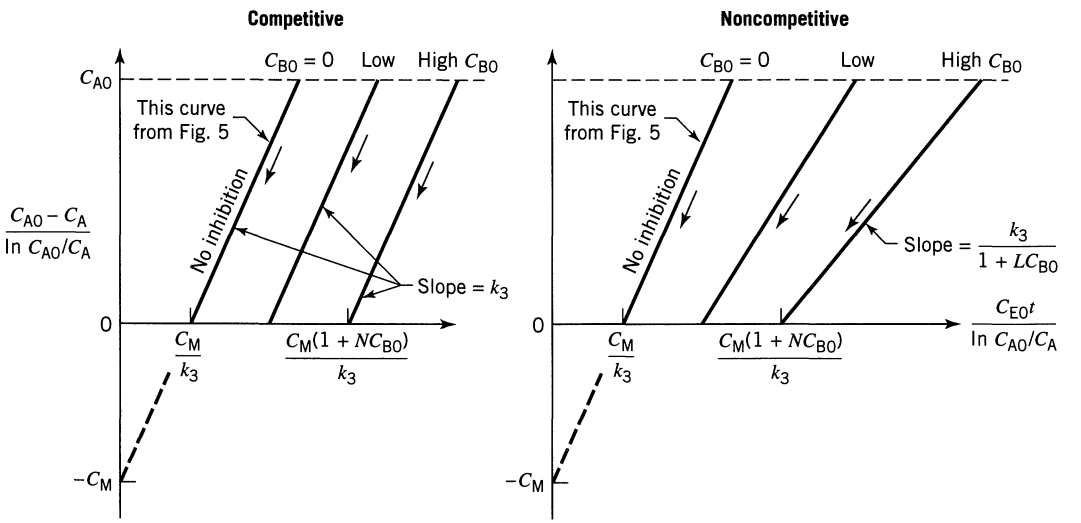


Figure 27.8 Effect of inhibition on batch or plug flow data.

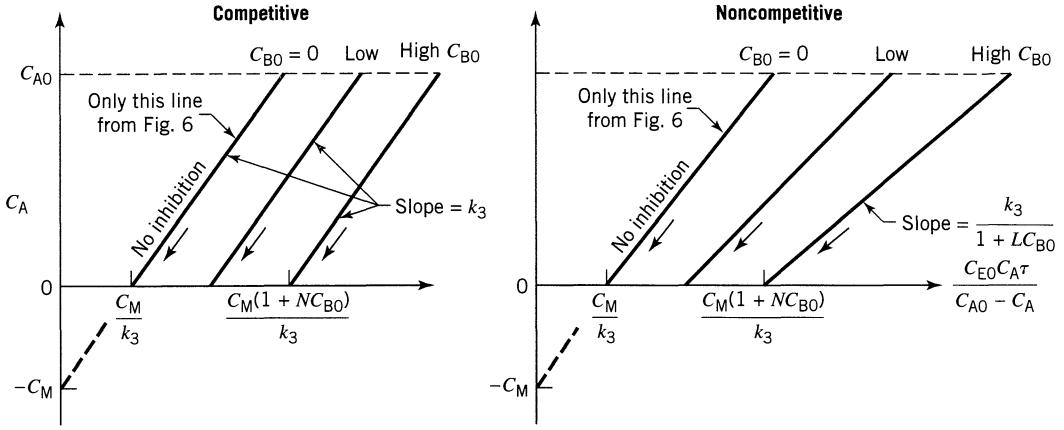


Figure 27.9 Effect of inhibition on mixed flow reactor data.

Comments

The M–M equation is the simplest expression to represent enzyme catalyzed reactions. It has been modified and extended in many ways. The two modes of inhibition introduced here are the simplest imaginable. Others are more complex to represent mathematically, so always try these first.

REFERENCE

Michaelis, L., and Menten, M. L., *Biochem. Z.*, **49,** 333 (1913).

PROBLEMS

27.1. Substrate A and enzyme E flow through a mixed flow reactor (V = 6 liter). From the entering and leaving concentrations and flow rate find a rate equation to represent the action of enzyme on substrate.

C_{E0}, mol/liter	C_{A0}, mol/liter	C_A, mol/liter	v, liter/hr
0.02	0.2	0.04	3.0
0.01	0.3	0.15	4.0
0.001	0.69	0.60	1.2

27.2. At room temperature sucrose is hydrolyzed by the enzyme sucrase as follows:

$$\text{sucrose} \xrightarrow{\text{sucrase}} \text{products}$$

Starting with sucrose (C_{A0} = 1 mol/m^3) and sucrase (C_{E0} = 0.01 mol/m^3) the following data are obtained in a batch reactor (concentrations are calculated from optical rotation measurements)

C_A, mol/m^3	0.68	0.16	0.006
t, hr	2	6	10

Find a rate equation to represent the kinetics of this reaction.

27.3. In a number of separate runs different concentrations of substrate and enzyme are introduced into a batch reactor and allowed to react. After a certain time the reaction is quenched and the vessel contents analyzed. From the results found below find a rate equation to represent the action of enzyme on substrate.

Run	C_{E0}, mol/m^3	C_{A0}, mol/m^3	C_A, mol/m^3	t, hr
1	3	400	10	1
2	2	200	5	1
3	1	20	1	1

27.4. Carbohydrate A decomposes in the presence of enzyme E. We also suspect that carbohydrate B in some way influences this decomposition. To study this phenomenon various concentrations of A, B, and E flow into and out of a mixed flow reactor (V = 240 cm^3).

(a) From the following data find a rate equation for the decomposition.
(b) What can you say about the role of B in the decomposition?
(c) Can you suggest a mechanism for this reaction?

C_{A0}, mol/m^3	C_A, mol/m^3	C_{B0}, mol/m^3	C_{E0}, mol/m^3	v, cm^3/min
200	50	0	12.5	80
900	300	0	5	24
1200	800	0	5	48
700	33.3	33.3	33.3	24
200	80	33.3	10	80
900	500	33.3	20	120

27.5. Enzyme E catalyzes the decomposition of substrate A. To see whether substance B acts as inhibitor we make two kinetic runs in a batch reactor, one with B present, the other without B. From the data recorded below

(a) Find a rate equation to represent the decomposition of A.
(b) What is the role of B in this decomposition?
(c) Suggest a mechanism for the reaction.

Run 1. $C_{A0} = 600$ mol/m^3, $C_{E0} = 8$ gm/m^3, no B present

C_A	350	160	40	10
t, hr	1	2	3	4

Run 2. $C_{A0} = 800$ mol/m^3, $C_{E0} = 8$ gm/m^3, $C_B = C_{B0} = 100$ mol/m^3

C_A	560	340	180	80	30
t, hr	1	2	3	4	5

Cellulose can be converted to sugar by the following enzymatic attack

$$\text{cellulase} \xrightarrow{\text{cellulase}} \text{sugar}$$

and both celluboise and glucose act to inhibit the breakdown. To study the kinetics of this reaction a number of runs are made in a mixed flow reactor kept at 50°C and using a feed of finely shredded cellulose (C_{A0} = 25 kg/m^3), enzyme (C_{E0} = 0.01 kg/m^3, same for all runs), and various inhibitors. The results are as follows:

Run	Exit Stream C_A, kg/m^3	*Series 1 no Inhibitor* τ, min	*Series 2 with Cellobiose* $C_{B0} = 5$ kg/m^3 τ, min	*Series 3 with Glucose* $C_{G0} = 10$ kg/m^3 τ, min
1	1.5	587	940	1020
2	4.5	279	387	433
3	9.0	171	213	250
4	21.0	36	40	50

27.6. Find a rate equation to represent the breakdown of cellulose by cellulase in the absence of inhibitor.

27.7. What is the role of cellubiose in the breakdown of cellulose (find the type of inhibition, and rate equation).

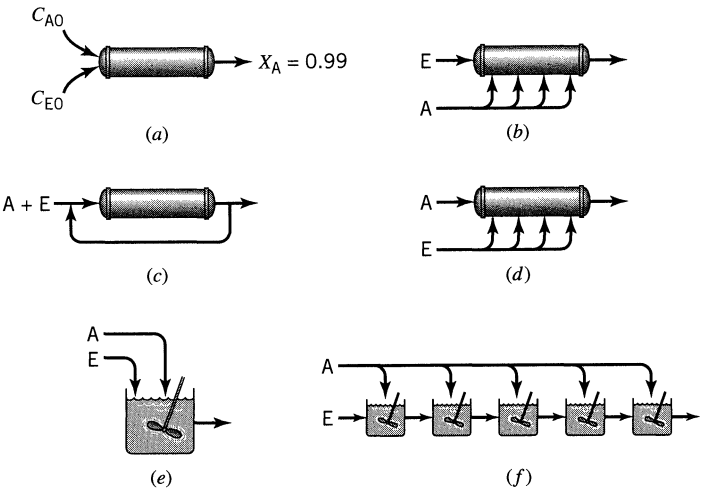
27.8. What is the role of glucose in the breakdown of cellulose (find the type of inhibition, and the rate equation).

The rate data for these problems are modified from Ghose and Das, *Advances in Biochemical Engineering,* **1,** 66 (1971).

27.9. Given the Michaelis–Menten rate form to represent enzyme-substrate reactions (or catalyst-reactant reaction)

$$A \xrightarrow{\text{enzyme}} R, \qquad -r_A = \frac{kC_AC_{E0}}{C_A + (\text{constant})}$$

which of the following contacting patterns when operated properly gives good reactor behavior (close to minimum reactor size) and which will not, if the reactor is to be fed two feed streams, one containing C_{A0}, the other C_{E0}?



Chapter 28

Microbial Fermentation—Introduction and Overall Picture

Natural fermentation is a complex situation with a hodge-podge of foods and cells all busily reacting. In this introduction let us only consider the very simplest of situations:

- one type of microbe C acting. We sometimes call this the cell or bug.
- one type of needed food A. This is called the substrate by life science workers.

If the food is right the bugs eat it, multiply, and in the process produce waste material R. In symbols

$$A \xrightarrow{C} C + R$$

In some cases the presence of product R inhibits the action of the cells, no matter how much food is available and we have what is called poisoning by product, or product poisoning. Wine making is an example of this

$$\begin{pmatrix}\text{squished grapes, fruit,}\\ \text{cereals, potatoes, etc.}\end{pmatrix} \xrightarrow{\text{bugs}} \begin{pmatrix}\text{more}\\ \text{bugs}\end{pmatrix} + \text{alcohol}$$

As the concentration of alcohol rises the cells multiply more slowly, and at about 12% alcohol the bugs quit. Alcohol is the poison here.

Activated sludge treatment of waste water is an example of a fermentation which is free of product poisoning

$$\begin{pmatrix}\text{organic waste}\\ \text{material}\end{pmatrix} \xrightarrow{\text{bugs}} \begin{pmatrix}\text{more}\\ \text{bugs}\end{pmatrix} + \begin{pmatrix}\text{breakdown products}\\ CO_2H_2O, \ldots\end{pmatrix}$$

Sometimes we are interested in the breakdown of A, as in waste water treatment. In other situations we are interested in producing cells C, as in the growing of yeast or single-cell protein for food. In still others we want the cell's waste material R, as in the production of penicillin and other antibiotics.

Let us see what typically happens with a single type of bug and a single food.

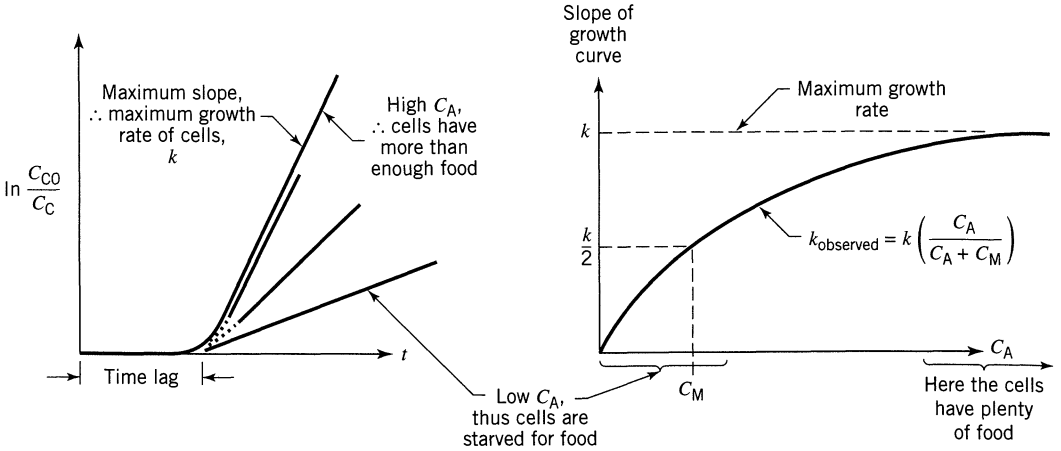


Figure 28.1 Cell growth in a uniform friendly environment.

Constant Environment Fermentation, Qualitative

What happens when we introduce a batch of microbes into a friendly constant composition medium having food of concentration C_A? First the microbes take some time to adapt to their new environment, then they grow exponentially. Thus we have the behavior shown in Fig. 28.1. Roughly—the time lag is a result of the "shock" to the cells in finding themselves in these new surroundings.

- the growth rate of cells (after the time lag) is given by Monod as

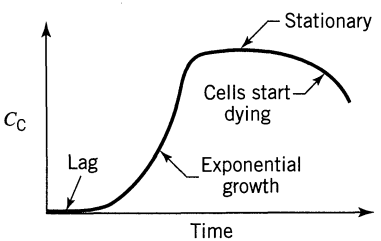
$$r_C = \frac{kC_A C_C}{C_A + C_M}$$

C_M: the concentration of A where the cells reproduce at 1/2 their maximum rate

Batch Fermentor, Qualitative

Here cells reproduce, the composition of the substrate changes, and product which can be toxic to the cells forms. Typically we see

- an induction period (time lag)
- a growth period
- a stationary period and
- a dying of cells



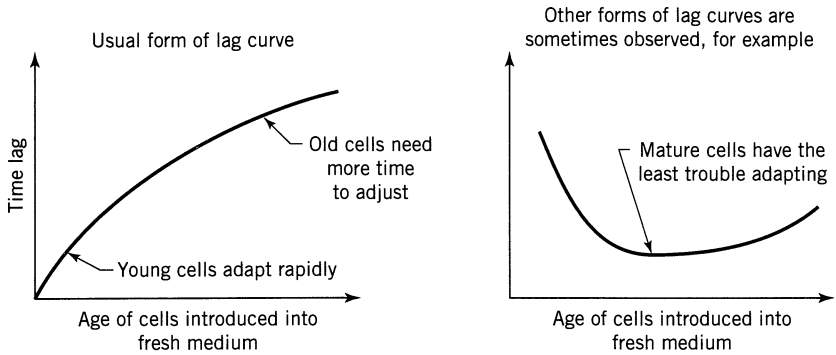


Figure 28.2 The time lag depends on the age of cells.

A few words follow about these regimes.

(a) Lag. As the cells in a container use up their food supply they stop multiplying, their enzyme activity decreases, low molecular weight chemicals diffuse out, and the cells change character. They age. So when they are introduced into a new environment a time lag is observed as the cells remanufacture the chemicals needed for growth and reproduction. In general any change in environment results in an induction period as the cells adjust. We find, as shown in Fig. 28.2.

(b) Growth and stationary phase. Cells grow exponentially in a uniform environment, but in a batch system the medium changes so the growth rate changes. The eventual drop in cell growth is governed either by

- depletion of food or
- accumulation of toxic materials (toxic to the cell).

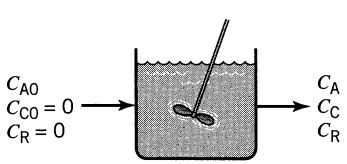
Graphically we summarize this in Fig. 28.3.

Mixed Flow Fermentor

Here the cells are in a uniform environment. No adaptation is needed and cell multiplication proceeds at a constant rate determined by the composition of the fluid in the vessel. This is frequently represented by a Monod-type equation

$$-r_C = \frac{kC_AC_C}{C_A + C_M}$$

The k value depends on all sorts of things: temperature, presence of trace elements, vitamins, toxic substances, light intensity, etc.



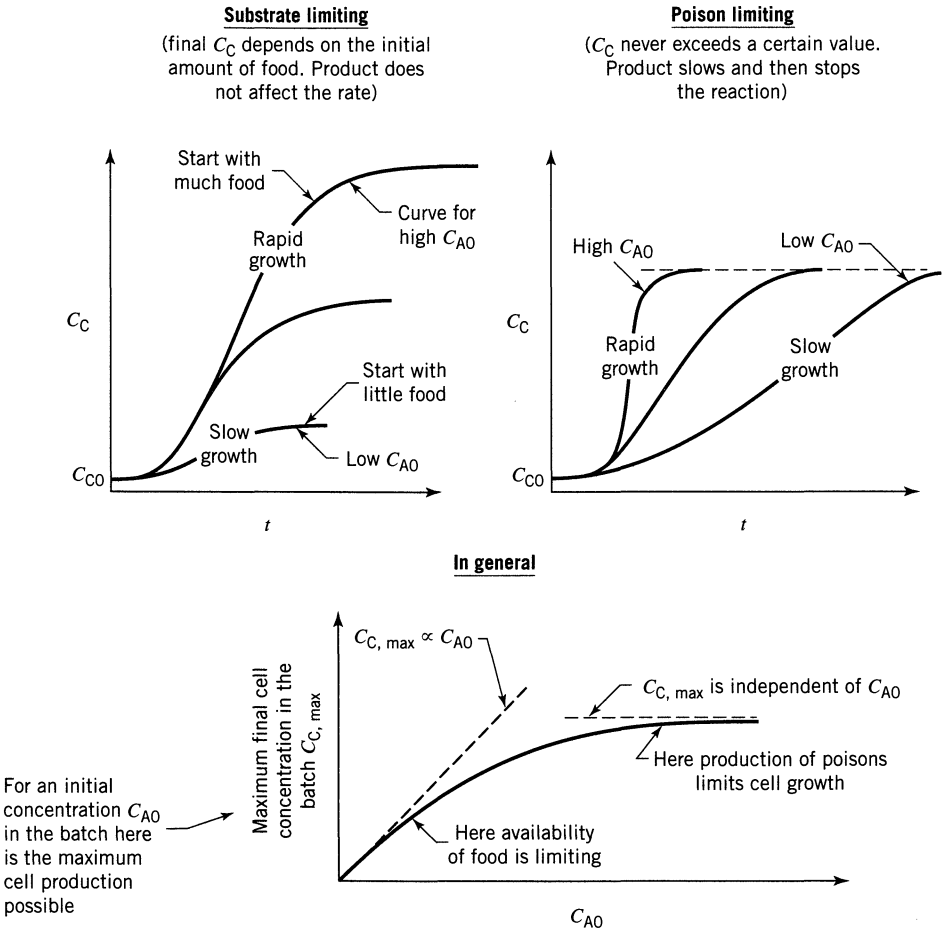


Figure 28.3 In a batch reactor the maximum cell production depends on the limiting mechanism.

Product Distribution and Fractional Yields

For the stoichiometric equation

$$A \xrightarrow{C} cC + rR$$

let us use the following shorthand notation for the instantaneous fractional yields

$$\left.\begin{aligned} \textcircled{C/A} &= \varphi(C/A) = \frac{d(\text{C formed})}{d(\text{A used})} \\ \textcircled{R/A} &= \varphi(R/A) = \frac{d(\text{R formed})}{d(\text{A used})} \\ \textcircled{R/C} &= \varphi(R/C) = \frac{d(\text{R formed})}{d(\text{C used})} \end{aligned}\right\} \quad (1)$$

Then we have the following relationships

$$\left.\begin{aligned} (R/A) &= (R/C)\cdot(C/A) \\ (A/C) &= 1/(C/A) \end{aligned}\right\} \tag{2}$$

and

$$\left.\begin{aligned} r_C &= (-r_A)\,(C/A) \\ r_R &= (-r_A)\,(R/A) \\ r_R &= (r_C)\,(R/C) \end{aligned}\right\} \tag{3}$$

In general the stoichiometry can be messy with fractional yields changing with composition. Treatment of that case can be difficult. We'd like therefore to make the simplification that all φ values remain constant at all compositions. This assumption may be reasonable for mixed flow, or for the exponential growth period of batch reactors, otherwise it is questionable.

Let us make this assumption anyway—all φ values stay constant. In this case for any change we can write

$$\left.\begin{aligned} C_C - C_{C0} &= (C/A)\,(C_{A0} - C_A) \quad \text{...or...} \quad C_C = C_{C0} + (C/A)\,(C_{A0} - C_A) \\ C_R - C_{R0} &= (R/A)\,(C_{A0} - C_A) \quad \text{...or...} \quad C_R = C_{R0} + (R/A)\,(C_{A0} - C_A) \\ C_R - C_{R0} &= (R/C)\,(C_C - C_{C0}) \quad \text{...or...} \quad C_R = C_{R0} + (R/C)\,(C_C - C_{C0}) \end{aligned}\right\} \tag{4}$$

Kinetic Expressions

The rate of cell multiplication depends in general on the availability of food and on the build up of wastes which interfere with cell multiplication. The simplest reasonable rate forms are shown below. We use these in the following chapters.

Availability of Food. For a reasonable quantitative expression make the analogy with enzyme kinetics.

For enzymes:

$$\left.\begin{aligned} A + E &\rightleftarrows X \\ X &\rightarrow R + E \\ \text{and } C_{E0} &= C_E + C_X \end{aligned}\right\} \quad \left.\begin{aligned} &\text{at high } C_A \quad \dots\ r_R = kC_{E0} \\ &\text{at low } C_A \quad \dots\ r_R = kC_{E0}\,C_A/C_M \\ &\text{at all } C_A \quad \dots\ r_R = \frac{kC_{E0}\,C_A}{C_A + C_M} \end{aligned}\right\} \tag{5}$$

Michaelis-Menten equation

M-M constant

For microbes:

$$\left.\begin{array}{l} A + C_{resting} \rightleftarrows C_{pregnant} \\ C_{pregnant} \rightarrow 2C_{resting} + R \\ \text{and } C_{total} = C_{pregnant} + C_{resting} \end{array}\right\} \quad \begin{array}{ll} \text{at high } C_A & \cdots\ r_R = kC_C \\ \text{at low } C_A & \cdots\ r_R = kC_C C_A / C_M \\ \text{at all } C_A & \cdots\ r_R = \dfrac{kC_C C_A}{C_A + C_M} \end{array} \quad (6)$$

Monod equation; Monod constant

Many other kinetic forms have been proposed and have been used in the past; however, they have all been forgotten since Monod came out with his expression. Its simplicity won the day. So we will use this type of expression throughout to relate the rate of cell growth to substrate concentration.

Effect of Harmful Wastes. As harmful wastes R build up, they interfere with cell multiplication. Thus, the observed Monod rate constant k_{obs} decreases with rise in C_R. A simple form of this relationship is

$$k_{obs} = k\left(1 - \frac{C_R}{C_R^*}\right)^n \quad (7)$$

k: rate constant in the absence of harmful waste material

n: order of product poisoning

where C_R^* is that concentration of R where all cell activity stops, in which case k_{obs} becomes zero. This expression is shown in Fig. 28.4.

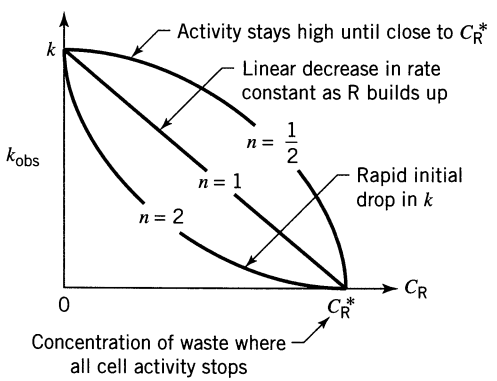


Figure 28.4 The observed k decreases as poisonous product R builds up (see Eq. 7).

General Kinetic Expression. The simplest expression of the Monod type which can account for both factors in microbial fermentation is

$$r_C = -r_A \,\textcircled{C/A}\, = r_R \,\textcircled{C/R}\, = k_{obs}\frac{C_A C_C}{C_A + C_M} \qquad (8)$$

generalized Monod equation

k_{obs} decreases as C_R rises

...where... $k_{obs} = k\left(1 - \frac{C_R}{C_R^*}\right)^n$

concentration where all reaction stops

In general, then, reaction and cell multiplication will slow down either by depletion of A (famine) or by build up of R (environmental pollution).

Planned Treatment of the Subject

The next two chapters treat in turn the performance expressions and design consequences for

- poison-free Monod kinetics. Here food limitation alone affects the growth rate of cells.
- product poisoning kinetics. Here some product formed during fermentation slows the rate.

We also assume in these chapters a constant fractional yield throughout, and since everything is liquid we take $\varepsilon_A = 0$, and we will use concentrations throughout.

More generally, an excess of either substrate or cells in the broth can also slow the rate of fermentation. In these situations the Monod equation should be suitably modified [see Han and Levenspiel, *Biotech. and Bioeng.*, **32,** 430 (1988), for a discussion and general treatment for all these forms of inhibition].

Chapter 29

Substrate-Limiting Microbial Fermentation

If we assume a constant fractional yield and no slowing of the rate as a result of product poisoning or increase in cell crowding in the broth, then the general rate equation of the previous chapter, Eq. 28.8, reduces to the well-known Monod equation

$$r_C = \text{(C/A)}(-r_A) = \frac{kC_A C_C}{C_A + C_M} \quad \text{...where} \quad C_C - C_{C0} = \text{(C/A)}(C_{A0} - C_A) \qquad \textbf{(1)}$$

(C_M: Monod constant)

and where C_{A0} and C_{C0} are the feed or starting compositions.

Let us examine ideal reactors processing feed which is reacting away according to these kinetics.

29.1 BATCH (OR PLUG FLOW) FERMENTORS

Consider the progress of this reaction. At the start C_{A0} is high, C_{C0} is low; at the end, $C_A \to 0$ while C_C is high. Thus, the rate at the beginning and at the end of a run is low, but it will be high at some intermediate composition. By putting $dr_C/dt = 0$, we find that the maximum rate occurs at

$$C_{A,\,\text{max rate}} = \sqrt{C_M^2 + C_M(C_{A0} + \text{(A/C)}\,C_{C0})} - C_M \qquad \textbf{(2)}$$

What this means is that with a feed C_{A0}, C_{C0} to any system, the key to proper design is to use mixed flow to reach $C_{A,\,\text{max rate}}$ all in one step, then use plug flow beyond this point. Thus, it is always important to know $C_{A,\,\text{max rate}}$.

Now let us return to our batch (or plug flow) reactor. Applying its performance equation we find

$$t_b = \tau_p = \int_{C_{C0}}^{C_C} \frac{dC_C}{r_C} = \frac{1}{k} \int_{C_{C0}}^{C_C} \frac{C_A + C_M}{C_C C_A}\, dC_C$$

$$C_{A0} - (A/C)(C_C - C_{C0})$$

$$= \int_{C_A}^{C_{A0}} \frac{dC_A}{(A/C) r_C} = \frac{1}{k} \int_{C_A}^{C_{A0}} \frac{C_A + C_M}{(A/C) C_C C_A}\, dC_A$$

$$C_{C0} + (C/A)(C_{A0} - C_A)$$

Integration then gives

$$kt_b = k\tau_p = \left(\frac{C_M}{C_{A0} + (A/C) C_{C0}} + 1 \right) \ln \frac{C_C}{C_{C0}} - \left(\frac{C_M}{C_{A0} + (A/C) C_{C0}} \right) \ln \frac{C_A}{C_{A0}} \qquad \textbf{(3)}$$

$$\text{... with } C_C - C_{C0} = (C/A)(C_{A0} - C_A)$$

If a time lag is involved just add t_{lag} to the above times to find t_{total}. If we wish we can write the performance equation in terms of C_R instead of C_A and C_C. Just remember that

$$C_R = C_{R0} + (R/C)(C_C - C_{C0}) = C_{R0} + (R/A)(C_{A0} - C_A) \qquad \textbf{(28.4)}$$

The sketches of Fig. 29.1 show the main properties of this performance equation.

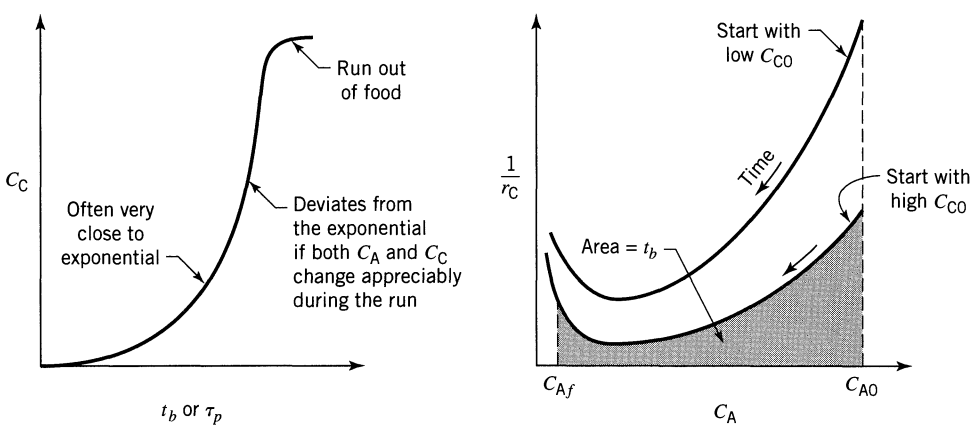


Figure 29.1 Behavior of batch or plug flow reactors for Monod type microbial fermentation.

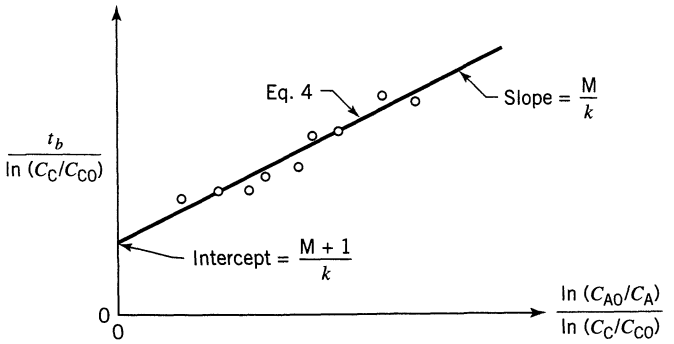


Figure 29.2 Evaluation of the Monod equation constants from batch data, method (a).

How to Find the Monod Constants from the Batch Experiment

Method (a). Rearrange Eq. 3 to give

$$\frac{t_b}{\ln(C_C/C_{C0})} = \frac{M+1}{k} + \frac{M}{k}\frac{\ln(C_{A0}/C_A)}{\ln(C_C/C_{C0})} \quad \text{with } M = \frac{C_M}{C_{A0} + (A/C)\,C_{C0}} \tag{4}$$

Then plot the data as shown in Fig. 29.2

Method (b). First find r_C by taking dC_C/dt data, then rearrange the Monod equation to give

$$\frac{C_C}{r_C} = \frac{1}{k} + \frac{C_M}{k}\frac{1}{C_A} \tag{5}$$

Then plot as shown in Fig. 29.3 to find C_M and k.

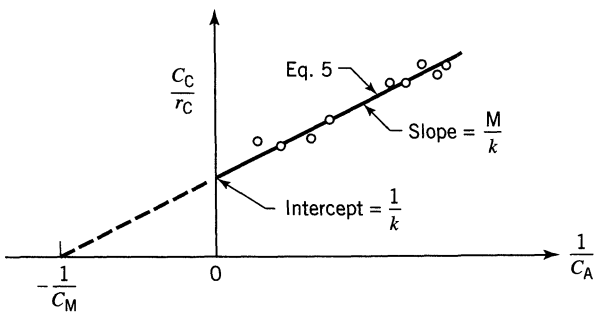


Figure 29.3 Evaluation of the constants of the Monod equation from batch reactor data, method (b).

Comments on Batch Operations

- Method (a) uses all the data directly and presents it as a linear plot. This method is probably better, all round.
- Method (b) requires taking derivatives or slopes from experimental data, is more tedious, and is probably less reliable.
- For high C_A, thus $C_A \gg C_M$, put $C_M = 0$ in the Monod equation. This gives $r_C = kC_C$, in which case the performance expression, Eq. 3, simplifies to

$$k\tau_p = \ln \frac{C_C}{C_{C0}} \qquad \text{... an exponential growth curve}$$

- For low C_A, thus $C_A \ll C_M$, the Monod equation becomes simple autocatalytic, and the performance expression, Eq. 3, reduces to

$$k\tau_p = \frac{C_M}{C_{A0} + (A/C)\, C_{C0}} \ln \frac{C_{A0}C_C}{C_A C_{C0}} \qquad \text{... S-shaped growth curve. See the autocatalytic equation in Chapter 3}$$

- For very high C_C the poison-free Monod equation just can't apply, for even if there is plenty enough food the cells will crowd out each other, and growth will slow down and will eventually stop. So, for very high cell concentration, we must go to product poison kinetics.
- It is awkward to try to evaluate the rate constants of the Monod equation from batch or plug flow data. Mixed flow data are so much simpler to interpret, as we shall see.

29.2 MIXED FLOW FERMENTORS

No Cells in Feed Stream, $C_{C0} = 0$

Assume Monod kinetics (no product poisoning), constant fractional yields φ, and no cells entering in the feed stream. Then the mixed flow performance equation becomes

$$\tau_m = \frac{\Delta C_i}{r_i} \qquad \text{where} \qquad i = \text{A, C, or R} \tag{6}$$

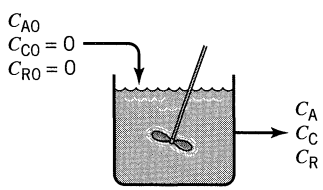


Figure 29*a*

Replacing r_i from Eq. 1 into Eq. 6 then gives

In terms of C_A

$$k\tau_m = \frac{C_M + C_A}{C_A} \quad \text{... or } C_A = \frac{C_M}{k\tau_m - 1} \quad \text{... for } k\tau_m > 1 + \frac{C_M}{C_{A0}}$$

In terms of C_C

$$k\tau_m = \frac{\textcircled{C/A}\,(C_{A0} + C_M) - C_C}{\textcircled{C/A}\,C_{A0} - C_C}$$

$$\text{... or } \quad C_C = \textcircled{C/A}\left(C_{A0} - \frac{C_M}{k\tau_m - 1}\right) \quad \text{... for } \quad k\tau_m > 1 + \frac{C_M}{C_{A0}} \tag{7}$$

In terms of C_R

$$k\tau_m = \frac{\textcircled{R/A}\,(C_{A0} + C_M) - C_R}{\textcircled{R/A}\,C_{A0} - C_R}$$

$$\text{... or } \quad C_R = \textcircled{R/A}\left(C_{A0} - \frac{C_M}{k\tau_m - 1}\right) \quad \text{... for } \quad k\tau_m > 1 + \frac{C_M}{C_{A0}}$$

no solution possible if $k\tau_m < 1 + \dfrac{C_M}{C_{A0}}$

This intriguing expression was developed by Monod (1949), and independently, at about the same time, by Novick and Szilard (1950).

To evaluate the kinetic constants from a set of mixed flow runs, rearrange Eq. 7 to give

$$\frac{1}{C_A} = \frac{k}{C_M}\tau_m - \frac{1}{C_M} \tag{8}$$

and the plot as shown in Fig. 29.4

From the performance equation we can show that everything—washout, optimum processing time, maximum production rate—all depend on C_M and C_{A0}, combined as follows:

$$N = \sqrt{1 + \frac{C_{A0}}{C_M}} \tag{9}$$

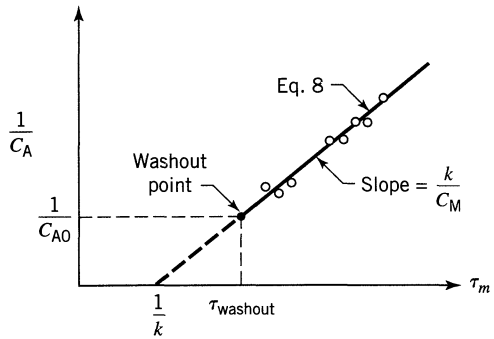


Figure 29.4 Evaluating the Monod constants from mixed flow reactor data.

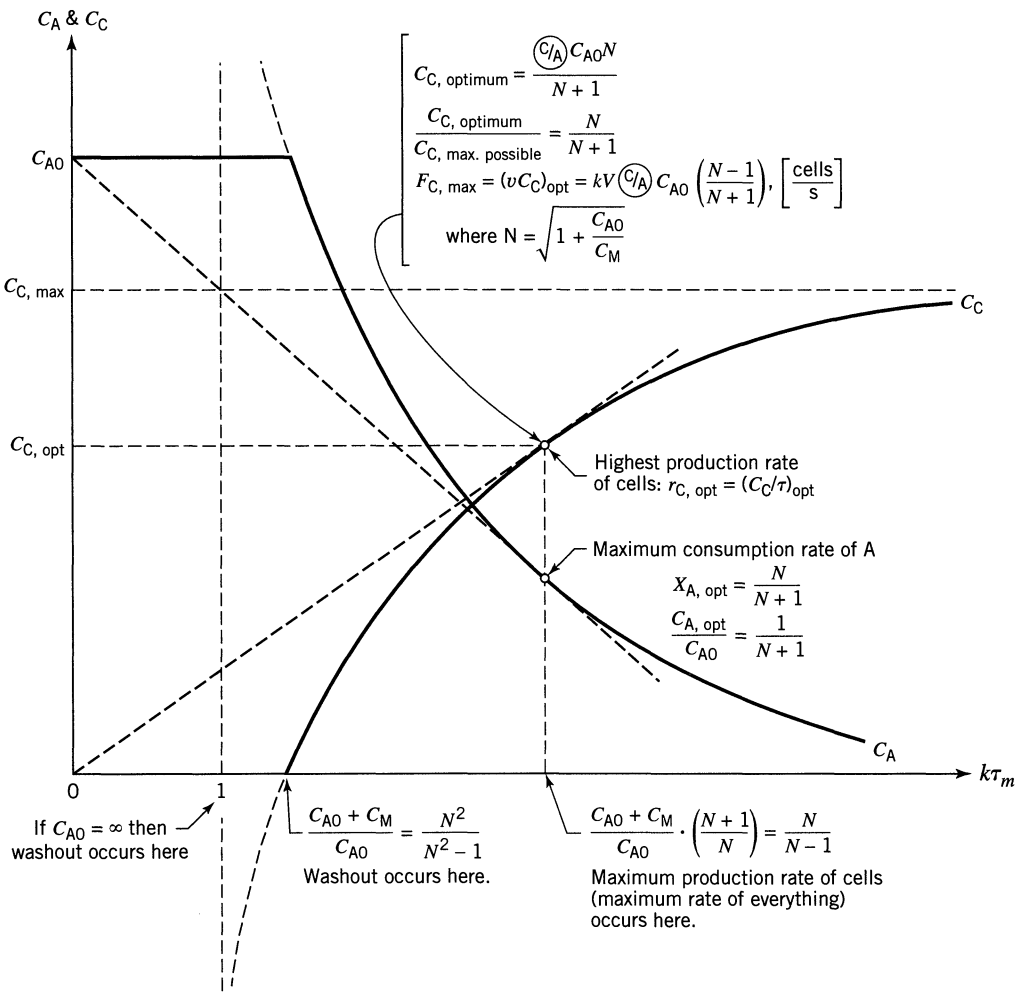


Figure 29.5 Summary of the mixed flow behavior of reactions which follow Monod kinetics.

Thus, optimum operations of a single mixed flow fermentor occurs when

$$\boxed{\frac{C_A}{C_{A0}} = \frac{1}{N+1}, \quad \frac{C_C}{C_{C,\,\text{max possible}}} = \frac{N}{N+1}, \quad k\tau_{\text{opt}} = \frac{N}{N-1}} \tag{10}$$

and washout occurs at

$$\boxed{k\tau_{\text{washout}} = \frac{N^2}{N^2-1}} \tag{11}$$

All this is shown in Fig. 29.5.

Feed Stream Contains Cells, $C_{C0} \neq 0$

With both feed and cells entering the fermentor, the Monod expression (Eq. 1) in the performance expression for the MFR (Eq. 6) gives

$$k\tau_m = \frac{(C_{A0} - C_A)(C_A + C_M)}{(A/C) \cdot C_{C0}C_A + C_A(C_{A0} - C_A)} \tag{12}$$

Examples 29.1*d*, 29.1*e* and 29.1*f* use this expression.

29.3 OPTIMUM OPERATION OF FERMENTORS

With poison-free Monod kinetics and a given feed, we have a U-shaped $1/r$ versus C curve, as shown in Fig. 29.6.

From Chapter 5 we have learned that with this form of rate-concentration curve we should operate as follows:

- To reach any point between A and B run part of the feed at A in mixed flow and mix with the rest of the feed.
- To reach any point between A and 0 go directly to point A in mixed flow, then use plug flow beyond A.

These two rules represent the key to optimum reactor behavior.

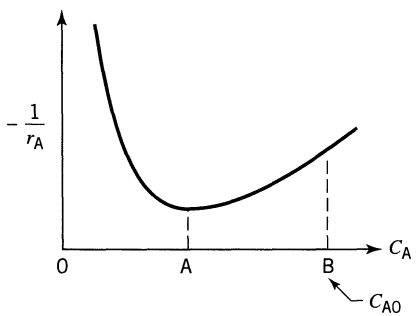


Figure 29.6 Rate-concentration behavior of Monod kinetics.

EXAMPLE 29.1 MIXED REACTORS FOR MONOD KINETICS

Let us illustrate the above important optimization principle by suggesting how to best operate various combinations of mixed flow reactors for a fermentation which follows Monod kinetics, and where

$$k = 2, \quad C_{A0} = 3, \quad C_M = 1, \quad \textcircled{C/A} = 1 \quad \text{and} \quad V_m = 1 \quad \text{for each reactor}$$

and all quantities are expressed in consistent units. Consider in turn the following setups

(a) A single MFR, with a feed rate $v = 3$
(b) A single MFR, with $v = 1$
(c) A single MFR, with $v = 1/3$
(d) Two MFR, $v = 3$
(e) Two MFR, $v = 1.5$
(f) Two MFR, $v = 0.5$

Do not calculate the outlet concentration of A or C.

SOLUTION

Preliminary. From the optimization rules for mixed flow reactions (see Eqs. 9 and 10), we have

$$N = \sqrt{1 + \frac{3}{1}} = 2$$

$$\tau_{m,\text{opt}} = \frac{N}{(N-1)k} = \frac{2}{(2-1)2} = 1$$

$$\tau_{m,\text{washout}} = \frac{N^2}{(N^2-1)k}$$

$$= \frac{4}{(4-1)2} = \frac{2}{3}$$

(a) High Feed Rate, Single Reactor, v = 3. If all the feed goes through the MFR the mean residence time $\tau = V/v = 1/3$ is too short and the cells don't stay in the reactor long enough and will wash out. So bypass some of the feed and let the reactor operate at its optimum, as shown in Fig. E29.1*a*.

(b) Intermediate Feed Rate, Single MFR, v = 1. Here if all the feed passes through the reactor $\tau = V/v = 1$, which is optimum, so operate that way, see Fig. E29.1*b*.

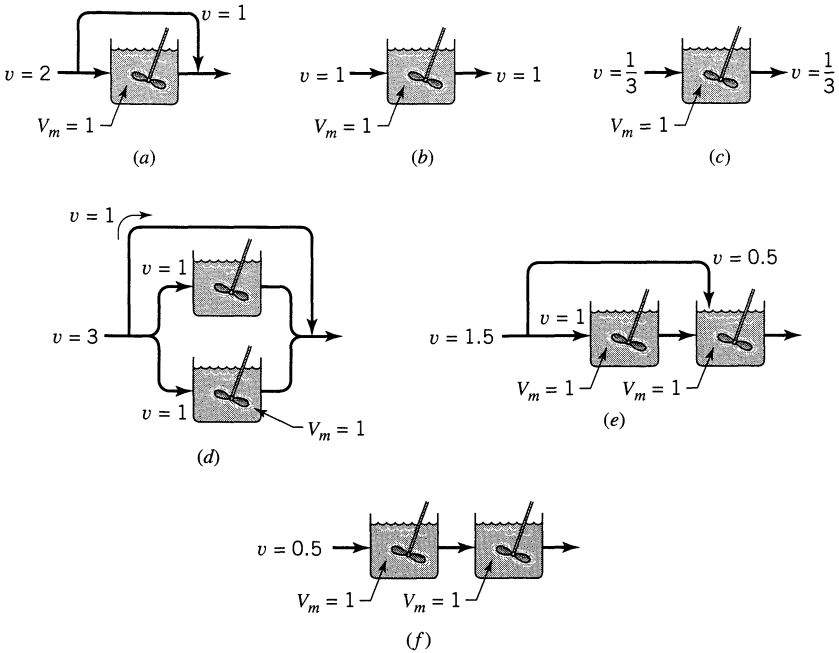


Figure E29.1

(c) Low Feed Rate, Single Reactor, v = 1/3. Here $\tau = V/v = 1/(1/3) = 3$, which is longer than the optimum, so pass everything through the reactor, as shown in Fig. E29.1*c*.

(d) High Feed Rate, 2 MFR, v = 3. Noting that for each MFR we want $\tau = 1$, which is optimum, we end up with the scheme shown in Fig. E29.1*d*.

(e) Intermediate Feed Rate, 2 MFR, v = 1.5. Here the best we can do is keep the first reactor at the best conditions, as shown in Fig. E29.1*e*.

(f) Low Feed Rate, 2 MFR, v = 0.5. Here the feed rate is too low to keep either reactor at the optimum so we end up with the scheme shown in Fig. E29.1*f*.

EXAMPLE 29.2 PLUG FLOW REACTOR FOR MONOD KINETICS

Let us extend Example 29.1 to the situation where we use a plug flow reactor (PFR) with or without recycle in place of mixed flow reactors (MFR). Find the optimum in the following situations.

(a) The volume of PFR $V_p = 3$, $v = 2$
(b) $V_p = 2$, $v = 3$

SOLUTION

First find the optimum conditions

$$N = 2, \quad \tau_{opt} = 1 \quad \text{and} \quad \tau_{washout} = \frac{2}{3}$$

(a) ***Low feed rate, $\tau = V_p/v = 3/2 = 1.5$.*** Here two optimal arrangements are shown in Fig. E29.2*a*.

(b) ***High feed rate, $\tau = V_p/v = 2/3$.*** If all the feed flows through the reactor we will have washout. So the optimum is as shown in Fig. E29.2*b*.

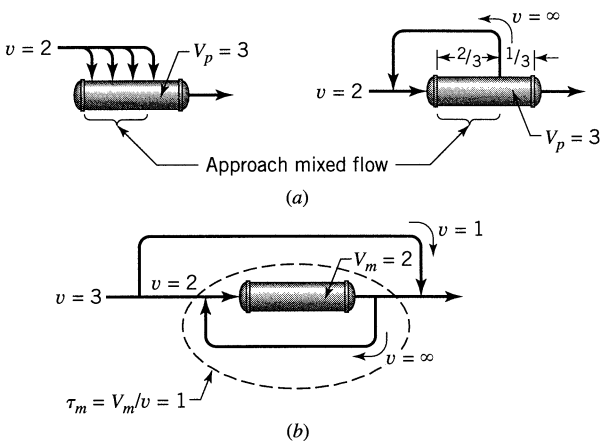


Figure E29.2

EXAMPLE 29.3 *GLUCOSE FOR E. COLI BACTERIA*

The *E. coli* microbe grows contentedly on glucose according to Monod kinetics as follows:

$$r_C = \frac{40\, C_A C_C}{C_A + 0.4} \frac{\text{kg cells}}{\text{m}^3 \cdot \text{hr}} \quad \text{with} \quad \begin{cases} \text{(C/A)} = 0.1 \\ C_A = \dfrac{\text{kg glucose}}{\text{m}^3} \end{cases}$$

What feed rate of glucose solution (C_{A0} = 6 kg/m^3) to a mixed flow reactor (V = 1 m^3) would give the maximum consumption rate of glucose, and maximum production rate of *E. coli* cells?

SOLUTION

To find the maximum consumption rate of glucose, first find N and then use the information given in Fig. 29.5.

$$N = \sqrt{1 + \frac{C_{A0}}{C_M}} = \sqrt{1 + \frac{6}{0.4}} = 4$$

$$k\tau_{opt} = \frac{N}{N-1} = \frac{4}{3} = 1.33$$

$$\therefore \tau_{opt} = \frac{1.33}{k} = \frac{1.33}{4} = 0.333 \text{ hr}$$

$$v_{opt} = \frac{V_m}{\tau_{opt}} = \frac{1}{0.333} = 3$$

The feed rate of glucose is

$$F_{A0} = (vC_{A0})_{opt} = (3)(6) = 18 \frac{\text{kg}}{\text{hr}}$$

The maximum consumption rate of glucose is

$$F_{A0}X_{A,opt} = 18\left(\frac{N}{N+1}\right) = 18\left(\frac{4}{5}\right) = \underline{\underline{14.4 \frac{\text{kg}}{\text{hr}}}}$$

The maximum production rate of *E. coli* is

$$F_{C\,max} = v_{opt}\, C_{C\,opt} = (3)\left[(C/A)C_{A0}\left(\frac{N}{N+1}\right)\right]$$

$$= (3)(0.1)(6)\left(\frac{4}{5}\right) = \underline{\underline{1.44 \frac{\text{kg}}{\text{hr}}}}$$

Comments

- The extension of the performance equations developed in this chapter to
  - plug flow with recycle of exit fluid
  - mixed flow with a feed which contains $C_{C0} \neq 0$
  - cell separation, concentration, and recycle

  and additional discussion are found in Levenspiel (1996) Chapter 83.
- The problems which follow quantitatively verify the findings of the first two examples.

- In the literature on continuous fermentation much effort is spent calculating what happens in this or that set up. Most of the schemes are nowhere near optimum so we do not consider them.
- When the φ values are constant throughout, at high and low conversions, then we have just one independent variable to deal with as composition changes with time or position. Thus, we can use any one concentration, C_A, C_R, or C_C in the performance expression. We can compare performance of various reactor types, plug flow, mixed flow, etc. with no difficulty. This is what we have done here.

  In general, however, $\varphi = f(C_A, C_R, C_C)$. And when φ varies with composition, then things get more difficult and we cannot directly compare reactor types.
- In 1939, as part of his thesis, Jacques Monod proposed the equation which we use here. The thesis was published as a book in 1948 and was later condensed and translated into English in 1949 (see end-of-chapter reference).
- In microbiology they use the terms
  - *substrate* for the feed.
  - *dilution rate* for $1/\tau$. In chemical engineering, we call this the space velocity. Here we use neither term. We use space time τ.
  - *chemostat, turbidostat* for mixed flow reactor.

  We should be aware of the difference in language.

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Monod J., *Ann. Rev. Microbiology,* **3,** 371 (1949)
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Novick, A., and Szilard, L., *Proc. N.A.S.,* Washington, **36,** 708 (1950).

PROBLEMS

29.1. A culture of *E. coli* was grown on lactose in a mixed flow reactor (V = 1 liter) using various flow rates of a C_{A0} = 160 mg lactose/liter feed. The following results were obtained:

v, liter/hr	C_A, mg/liter	Cell Concentration, Arbitrary
0.2	4	15.6
0.4	10	15
0.8	40	12
1.0	100	6

Find a rate equation to represent this growth.

E. coli lives and grows on mannitol with the following kinetics

$$r_C = \frac{1.2\, C_A C_C}{C_A + 2},\quad C_A = \text{gm mannitol/m}^3,\quad \textcircled{C/A} = 0.1 \text{ gm cells/gm mannitol}$$

Find the outlet concentration of cells from the reactor when 1 m^3/hr of mannitol solution (C_{A0} = 6 gm/m^3) is fed directly to a mixed flow reactor of volume

29.2. V_m = 5 m^3 **29.3.** V_m = 1 m^3.

Can you do better and produce more cells (if so, find C_C) by proper bypass or recycle of fluid from the reactor for the system of

29.4. problem 2? **29.5.** problem 3?

29.6. How curious—two different flow rates of C_{A0} = 500 mol/m^3 feed to our 1 m^3 mixed flow reactor produces the same 100 gm/hr of yeast cells in the exit stream, namely

- at 0.5 m^3/hr of feed for which we find C_A = 100 mol/m^3
- at 1 m^3/hr of feed for which we find C_A = 300 mol/m^3

Substrate limiting Monod kinetics should well represent yeast formation. From this information, find

(a) the fractional yield of yeast,
(b) the kinetic equation for yeast formation,
(c) the flow rate for maximum yeast production,
(d) the maximum production rate of yeast.

A stream of reactant A (C_{A0} = 3, C_{R0} = 0, C_{C0} = 0) is to be decomposed by the following microbial fermentation

$$A \rightarrow R + C,\quad r_C = \frac{k C_A C_C}{C_A + C_M} \quad \text{with} \begin{cases} k = 2 \\ C_M = 1 \\ \textcircled{C/A} = 0.5 \end{cases}$$

In the following problems sketch your recommended reactor setup with recycle, bypass, etc., and on each sketch indicate pertinent quantities.

What is the lowest C_A which can be obtained in a single mixed flow reactor of size V_m = 1 for a feed rate of

29.7. v = 1/3 **29.8.** v = 3

What is the lowest C_A which can be obtained with two properly connected mixed flow reactors, each of volume $V = 1$, for a feed rate

29.9. $v = 2$ **29.10.** $v = 1$

What is the lowest C_A which can be obtained with three wisely connected mixed flow reactors, each of volume $V_m = 1$, for a feed rate

29.11. $v = 6$ **29.12.** $v = 2$

For a feed rate $v = 3$ what is the smallest size of plug flow reactor with appropriate piping (bypass or recycle or slide taps on the reactor) which will give

29.13. $C_C = 0.5$, side tap allowed

29.14. $C_C = 1.25$, side tap not allowed

29.15. $C_C = 1.44$, side tap allowed

29.16. Find the lowest C_A obtainable from a plug flow reactor of volume $V_p = 4$ (bypass, recycle and/or side taps are all allowed) for a feed rate $v = 6$

29.17. Given the Monod equation to represent a microbial fermentation

$$A \xrightarrow{\text{cells, C}} R + C \qquad -r_A = \frac{kC_AC_C}{C_A + \text{constant}}$$

Which of the contacting patterns of Fig. P29.17 could be optimum, and which can never be? By optimum we mean minimizing reactor volume for a feed consisting only of C_{A0}.

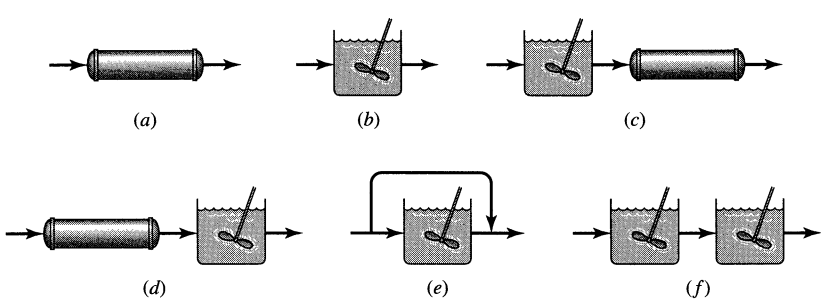

Figure P29.17

29.18. In his thesis, which was published as a book in 1948, Monod first proposed the celebrated equation that bears his name. As experimental support for this equation he presented results from four batch reactor runs on the

growth of a pure bacterial culture in a lactose solution (see Monod, 1958, p. 74). Here are the reported data for one of his runs.

Time Interval Number	Δt, hr	$\overline{C}_A$	C_C
1	0.54	137	15.5 to 23.0
2	0.36	114	23.0 to 30.0
3	0.33	90	30.0 to 38.8
4	0.35	43	38.8 to 48.5
5	0.37	29	48.5 to 58.3
6	0.38	9	58.3 to 61.3
7	0.37	2	61.3 to 62.5

Fit the Monod equation to this data.

29.19. In Example 29.1*e* we could have used any one of the three contacting schemes shown in Fig. P29.19. We stated without proof that the bypass scheme was best. Show that this is so by calculating C_{Aout} for the three contacting schemes of Fig. P29.19.

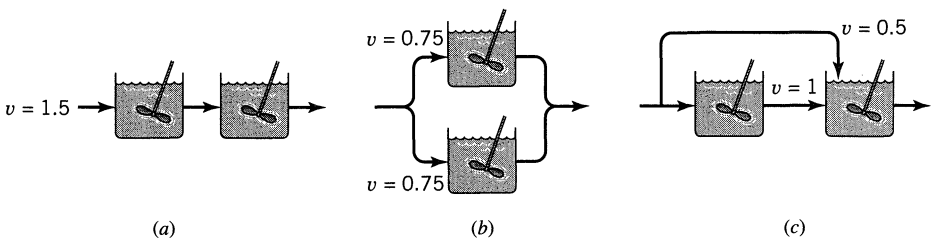

Figure P29.19

Chapter 30

Product-Limiting Microbial Fermentation

With sufficient food and harmonious environment, cells multiply freely. However, no matter how much food is available, eventually either cells crowd each other out or their waste products inhibit their growth. We call this *product poisoning.* Hence, Monod kinetics always is a special case of a more general rate form which includes product poisoning. A simple equation of the general rate form for this situation is

$$r_C = \text{(C/R)}\, r_R = k\left(1 - \frac{C_R}{C_R}\right)^n \frac{C_A C_C}{C_A + C_M} \qquad (1)$$

order of product poisoning

rate constant in a poison-free environment

k_{obs} decreases as product builds up

In the special case of sufficient food, or $C_A \gg C_M$, and $n = 1$, the above equation reduces to the simplest expression for product poison control

$$r_C = \text{(C/R)}\, r_R = k\left(1 - \frac{C_R}{C_R^*}\right) C_C \qquad (2)$$

reaction stops when C_R reaches C_R^*

We start with the rate form of Eq. 2, then we extend the treatment to systems where $n \neq 1$, or Eq. 1. Let us also develop everything in terms of C_R, in which case Eq. 2 becomes

$$r_R = \text{(R/C)}\, r_C = \text{(R/C)}\, k\left(1 - \frac{C_R}{C_R^*}\right) C_C = k\left(1 - \frac{C_R}{C_R^*}\right) (C_R - C_{R0} + \text{(R/C)}\, C_{C0}) \qquad (3)$$

The maximum rate then occurs where $\frac{dr_R}{dC_R} = 0$. Solving gives

$$C_{R,\,\text{max rate}} = \frac{1}{2}\left(C_{R0} + C_R^* - \textcircled{R/C}\, C_{C0}\right) \tag{4}$$

Thus, there always is a composition where the rate is optimum.

30.1 BATCH OR PLUG FLOW FERMENTORS FOR $n = 1$

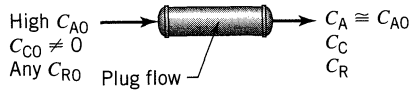

Figure 30*a*

To relate time to concentration, we integrate the performance expression of Eq. 3

$$t_p = \tau_b = \int_{C_{R0}}^{C_R} \frac{dC_R}{r_R} = \int_{C_{R0}}^{C_R} \frac{dC_R}{k\left(1 - \dfrac{C_R}{C_R^*}\right)\left(C_R - C_{R0} + \textcircled{R/C}\, C_{C0}\right)}$$

or in terms of product R,

$$k\tau_p = k\tau_b = \frac{C_R^*}{C_R - C_{R0} + \textcircled{R/C}\, C_{C0}} \ln \frac{C_C(C_R^* - C_{R0})}{C_{C0}(C_R^* - C_R)} \tag{5}$$

($C_C = C_{C0} + \textcircled{C/R}\,(C_R - C_{R0})$)

Graphically this performance equation is shown in Fig. 30.1.

Since the $1/r_R$ versus C_R curve is U-shaped, the optimum way of running the plug flow reactor is shown in Fig. 30.2.

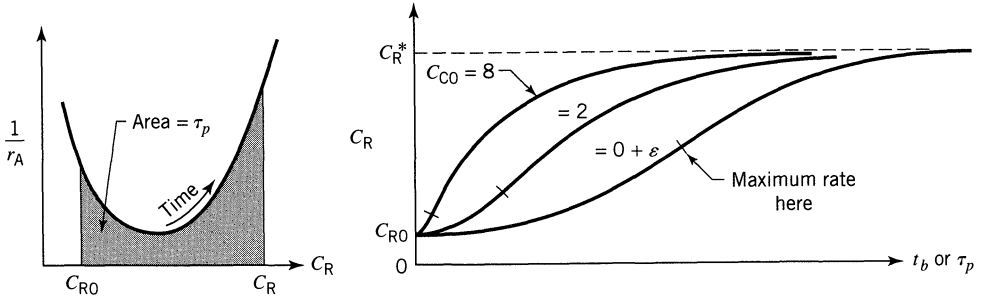

Figure 30.1 Graphical representation of Eq. 5.

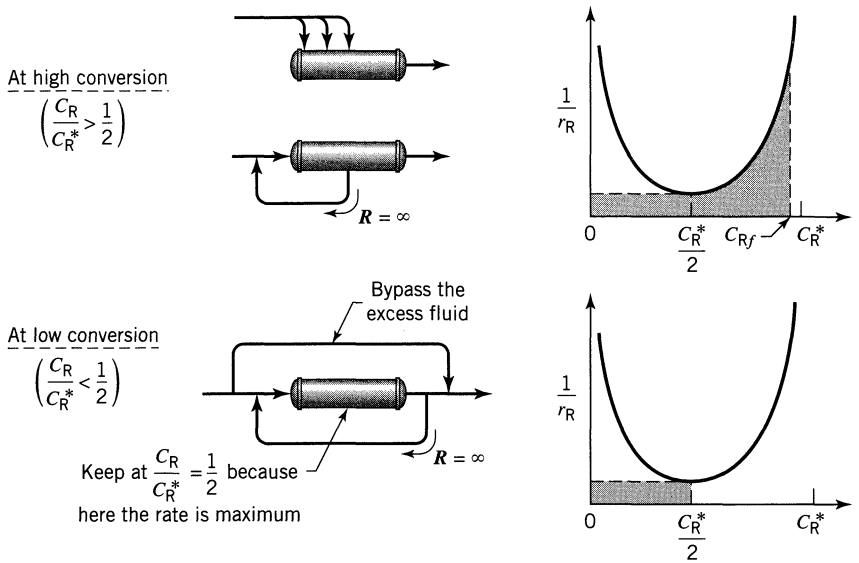

Figure 30.2 Optimum operation of a plug flow fermentor for poison limiting kinetics.

30.2 MIXED FLOW FERMENTORS FOR $n = 1$

For the case of negligible time lag for the feed cells which enter their new environment, and for high C_{A0} we have

$$\tau_m = \frac{C_R - C_{R0}}{r_R} = \frac{C_R - C_{R0}}{k\left(1 - \frac{C_R}{C_R^*}\right)\left(C_R - C_{R0} + \text{(R/C)}\, C_{C0}\right)} \tag{9}$$

For the special case where $C_{C0} = 0$ and $C_{R0} = 0$ the above general expression simplifies to

$$\boxed{k\tau_m = \frac{C_R^*}{C_R^* - C_R} = \frac{1}{1 - \frac{C_R}{C_R^*}}} \quad \text{for} \quad k\tau_m > 1 \tag{10}$$

To evaluate the kinetic constants from mixed flow experiments rearrange Eq. 10 as in Eq. 11 and plot as shown in Fig. 30.3.

$$C_R = C_R^* - \frac{C_R}{k} \cdot \frac{1}{\tau_m} \tag{11}$$

The properties of Eq. 10 are displayed in Fig. 30.4.

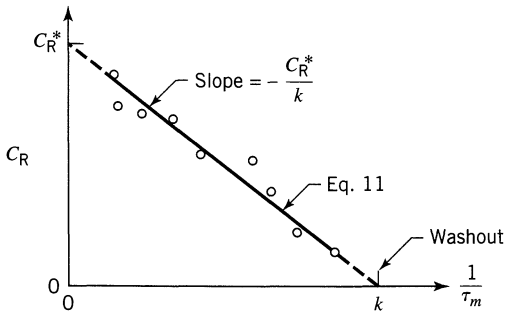

Figure 30.3 Evaluation of the rate constants of Eq. 2 from data taken in a mixed flow reactor.

Comments

For mixed flow with $C_{C0} = 0$, $C_{R0} = 0$, and any high C_{A0}.

- washout occurs at $k\tau_m = 1$ for any feed
- maximum production rate of cells and product R is obtained at

$$k\tau_m = 2 \ldots \text{and} \ldots C_R = C_R^*/2$$

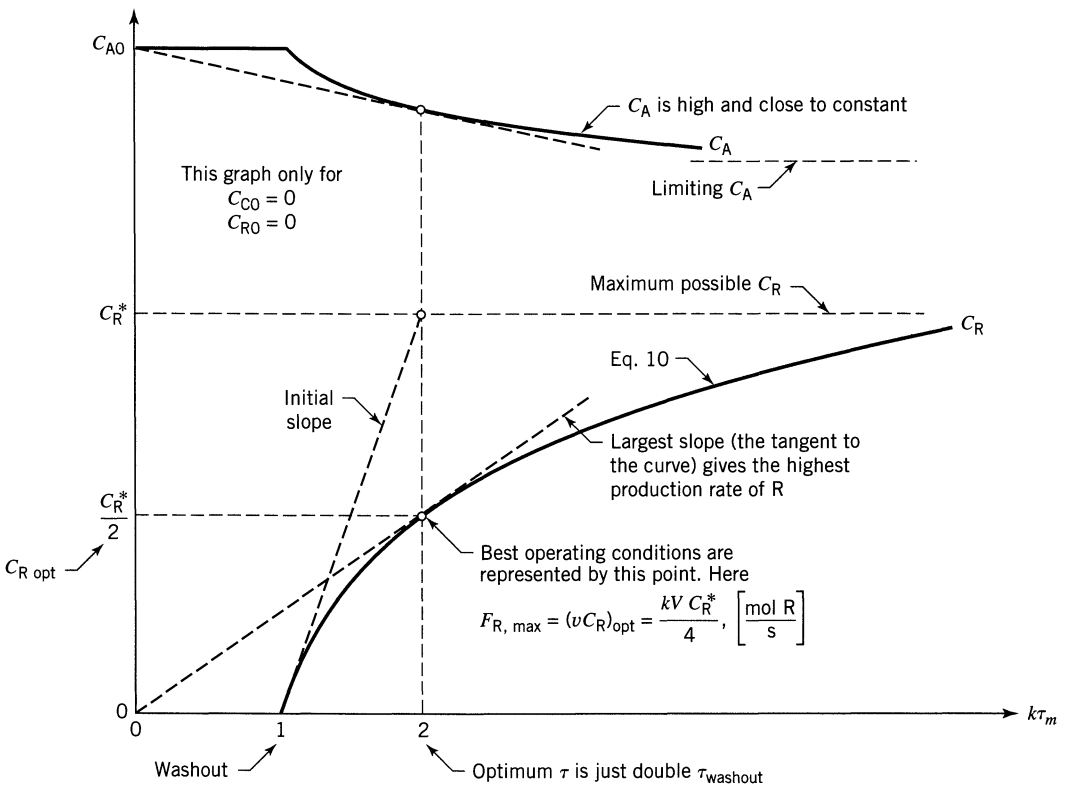

Figure 30.4 Properties of the mixed flow equation for the kinetics of Eq. 2.

- the maximum production rate of cells and product is found to be

$$F_{R,\max} = \text{(R/C)}\, F_{C,\max} = kVC_R^*/4$$

- the C_C curve is similar in shape to the C_R curve and is proportional to it. Thus, it rises from 0 to (C/R) C_R^*.

- Optimum operations for multistage systems follow the same pattern as for poison-free systems. The general rule is to use mixed flow to reach the maximum rate in one step. Proceed beyond this with plug flow.
  Note that the maximum rate occurs at $C_R^*/2$ when $C_{C0} = 0$ and $C_{R0} = 0$.

Fermentation with $n \neq 1$ Poison Limiting Kinetics

For n-th order product poisoning kinetics the rate equation is

$$-r_R = k\left(1 - \frac{C_R}{C_R^*}\right)^n C_C \tag{12}$$

These kinetics are displayed in Fig. 30.5. The performance equation for plug flow is quite messy; however, for mixed flow the performance equation can be obtained directly. Thus in general, for $C_{C0} \neq 0$ and $C_{R0} \neq 0$ we have

$$k\tau_m = \frac{C_R - C_{R0}}{\left(C_R - C_{R0} + \text{(R/C)}\, C_{C0}\right)\left(1 - \dfrac{C_R}{C_R^*}\right)^n} \tag{13}$$

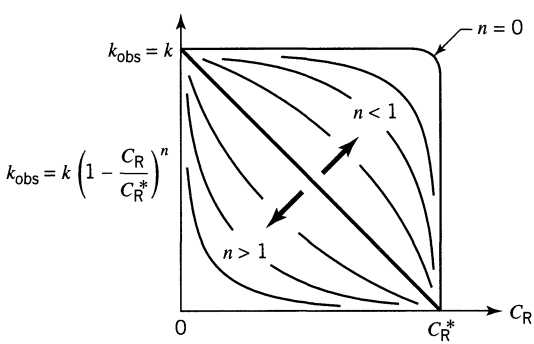

Figure 30.5 Reaction slowdown is strongly dependent on the order of poisoning, n.

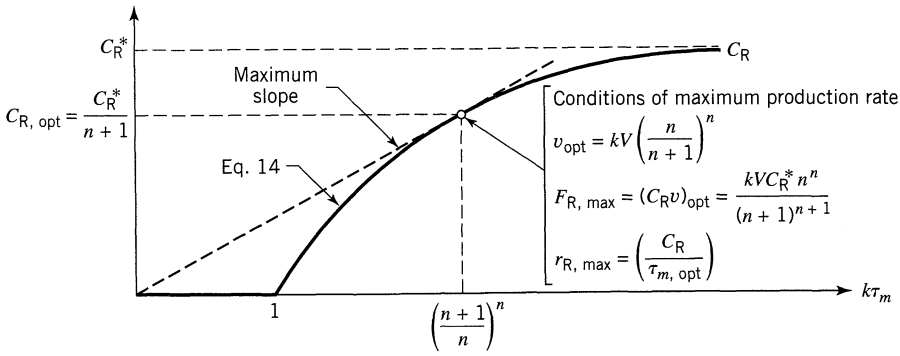

Figure 30.6 Behavior of a mixed flow reactor for the poison influenced kinetics of Eq. 12.

and for the special case where $C_{C0} = 0$ and $C_{R0} = 0$

$$k\tau_m = \frac{1}{\left(1 - \frac{C_R}{C_R^*}\right)^n} \qquad \text{when} \qquad k\tau_m > 1 \tag{14}$$

The properties of this equation, washout, maximum production, etc. are displayed in Fig. 30.6. To find the kinetic constants C_R^*, k and n from experiment first evaluate C_R^* in a batch run using an excess of reactant A and letting $t \to \infty$. Then rearrange the mixed flow performance equation to give

$$\log \tau_m = -\log k + n \log \left(\frac{C_R^*}{C_R^* - C_R}\right) \tag{15}$$

and plot as shown in Fig. 30.7. This will give the kinetic constants k and n.

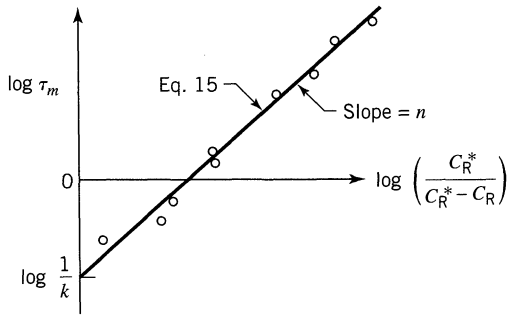

Figure 30.7 Finding the order of product poisoning and the rate constant of Eq. 12 from mixed flow reactor data.

Discussion

The similarity in shape of the mixed flow graphs for product-limiting and substrate-limiting (Monod) kinetics (Fig. 29.5 and Fig. 30.4), has led many investigators to fit product poisoning systems with the simple Monod equation. The fit will be good, but don't try to use the fitted equation for different feed conditions. Your predictions are likely to be way off because the logic of the extension is wrong.

You must first find which of these two factors is rate limiting. This is easy to do, so there is no excuse for using the wrong expression. In one case the final extent of reaction is dependent on C_{A0} and not on C_{R0}; in the other case just the opposite holds. The discussion and sketches of Chapter 28 show this.

Expressions for related situations such as plug flow with recycle, and where both substrate and product both affect the rate are developed by Levenspiel (1996) Chapter 84.

EXAMPLE 30.1 *FRUIT FLY COCKTAIL*

Crushed fruit flies (A) ferment to produce an aromatic alcoholic drink (R) with product-limiting kinetics estimated as follows:

$$\left.\begin{aligned} &A \xrightarrow{C} R + C \\ &r_R = k\left(1 - \frac{C_R}{C_R^*}\right)^n C_C \end{aligned}\right\} \text{with} \begin{cases} k = \sqrt{3}, \text{hr}^{-1} \\ n = 1 \text{ in a springtime operation} \\ C_R^* = 0.12 \text{ kg alc/kg solution} \\ \rho = 1000 \text{ kg/m}^3 \end{cases}$$

What is the most alcohol you can produce (kg/hr) in a commercial-sized mixed flow reactor ($V_m = 30$ m^3)? Also calculate the concentration of alcohol in the cocktail, and the feed rate needed of fragrant fresh frappéed fluit fries.

SOLUTION

From the given data and from Fig. 30.4, we find the conditions which yield the optimum. Thus

$$C_R^* = \left(\frac{0.12 \text{ kg alc}}{\text{kg sol}}\right)\left(\frac{10^3 \text{ kg sol}}{\text{m}^3 \text{ sol}}\right) = 120 \text{ kg/m}^3$$

$$\therefore C_{R,\text{opt}} = \frac{C_R^*}{2} = \left(\frac{120 \text{ kg alc}}{\text{m}^3}\right)\frac{1}{2} = 60\frac{\text{kg alc}}{\text{m}^3} = \underline{\underline{6\% \text{ alcohol}}}$$

Again, from Fig. 30.4 we find

$$k\tau_{\text{washout}} = 1 \qquad \therefore \tau_{\text{washout}} = \frac{1}{\sqrt{3}} \text{hr}$$

and

$$\tau_{opt} = 2\tau_{washout} = \frac{2}{\sqrt{3}}\,\text{hr}$$

But $\tau_{opt} = \dfrac{V}{v_{opt}}$ so the optimum feed rate is

$$v_{opt} = \frac{V}{\tau_{opt}} = \frac{30\sqrt{3}}{2} = \underline{\underline{25.98\ \text{m}^3/\text{hr}}}$$

The production rate of alcohol, again from Fig. 30.4, is

$$F_R = v_{opt} \cdot C_{R,opt} = (25.98)\,(60) = \underline{\underline{1558\ \text{kg alc/hr}}}$$

■

REFERENCES

Levenspiel, O., *Chemical Reactor Omnibook,* Chapter 84, OSU Bookstores, Corvallis, OR, 1996.

PROBLEMS

Material R is to be produced from the following microbial fermentation, using a feed stream $C_{A0} = 10^6$, $C_{R0} = 0$, $C_{C0} = 0$. All quantities are given in consistent SI units.

$$\begin{aligned} &A \xrightarrow{C} R + C \qquad \text{with} \begin{cases} k = 2 \\ C_M = 50 \\ C_R^* = 12 \\ (R/A) = 0.1 \\ (C/A) = 0.01 \end{cases} \\ &r_C = k\left(1 - \frac{C_R}{C_R^*}\right)\frac{C_A C_C}{C_A + C_M} \end{aligned} \tag{9}$$

In each of the following problems sketch your recommended reactor setup and on the sketch indicate pertinent quantities.

What C_R is obtainable in a single mixed flow reactor of size $V_m = 1$ for a feed rate

30.1. . . . $v = 1$ **30.2.** . . . $v = 4$

What C_R is obtainable using two mixed flow reactors, each of volume $V_m = 1$, for a feed rate

30.3. . . . $v = 1$ **30.4.** . . . $v = 3$

For a feed rate $v = 3$ what size of plug flow reactor with appropriate piping, recycle, bypass or anything you want to use is needed to give

30.5. . . . $C_R = 6$ **30.6.** . . . $C_R = 4$ **30.7.** . . . $C_R = 9$

30.8. The microbial fermentation of A produces R as follows

$$10A + \text{Cell} \rightarrow 18R + 2 \text{ Cells}$$

and experiments in a mixed flow reactor with $C_{A0} = 250$ mol/m^3 show that

$$C_R = 24 \text{ mol/m}^3 \text{ when } \tau = 1.5 \text{ hr}$$
$$C_R = 30 \text{ mol/m}^3 \text{ when } \tau = 3.0 \text{ hr}$$

In addition, there seems to be a limiting upper value for C_R at 36 mol/m^3 for any τ_m, C_A, or C_C.

From this information determine how to maximize the fractional yield of R, or (R/A), from a feed stream of 10 m^3/hr of $C_{A0} = 350$ mol/m^3. Cell or product separation and recycle are not practical in this system, so only consider a once-through system. Present your answer as a sketch showing reactor type, reactor volume, C_R in the exit stream, and the moles of R produced/hr.

In spring the fermentation of fruit flies proceeds with $n = 1$ kinetics, as shown in Example 30.1. However, in winter or summer, maybe because of a difference in temperature, the poisoning proceeds differently. So repeat Example 30.1 with one change:

30.9. in winter, $n = \frac{1}{2}$ **30.10.** in summer, $n = 2$

Note: To tell how the value of n affects the reactor performance, compare your answers here with the answer in Example 30.1.

30.11. Shredded chemical reactor textbooks are degraded to glucose in a pilot plant well stirred fermenter ($V_m = 50$ lit) under the action of a word gobbling bug. With a large excess of these shredded incomprehensible words, the presence of glucose becomes the rate limiting factor. We summarize the findings as follows

when $v = 16$ books/hr	$C_R = 54$ μmol/liter
when $v = 4$ books/hr	$C_R = 75$ μmol/lit
when $v \rightarrow 0$	$C_R \rightarrow 90$ μmol/lit

Determine the flow rate of books which maximizes glucose production per word gobbled up, and find this production rate.

30.12. Professor Microbe has submitted a paper for publication in which he studied the growth of a new strain of bug in a mixed flow fermenter ($V_m = 46.4$) using a pure substrate feed ($C_{A0} = 150$, $C_{R0} = C_{C0} = 0$). His raw data is as follows

v	C_A	
4.64	5	
20.0	125	with (R/A) = 0.5
22.0	150 (washout)	

He asserts, without giving details, that this data clearly represents poison-limiting kinetics with rate constants

$$k = 0.50, \quad C_R^* = 90.6, \quad n = 1.0$$

The reviewer of the paper, Dr. Ferment, counters that Microbe is quite wrong, that the data in fact represents substrate limiting Monod kinetics with

$$C_M = 20, \qquad k = 0.50$$

But, out of orneriness, he didn't present the details of his calculations either. The editor can't determine who is right (this is not his field), so he sends the paper and the review to duWayne Zuelhsdorff. What is duWayne's answer? Is Microbe, or Ferment, or both, or neither, right?