

Basic Bioreactor Design

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Preface

Basic Bioreactor Design is based on the course material of the graduate course in biochemical engineering at Wageningen Agricultural University. This course has two objectives:

- 1 To provide the basic principles of reactor design
- 2 To select the relevant principles and data for practical process engineering purposes

These objectives determined the scope of material covered in this book. A limited number of reactor types as well as a limited number of phenomena are discussed. Yet we assume that the ones dealt with are enough to solve 95% of the problems and questions encountered in commercial fermentation. The book is intended for two groups of people: first, graduate students, to use as a textbook for learning the basic principles and methods of bioreactor design, and second, company engineers and biotechnologists, to use it as a handbook of fermenter design and engineering.

Part of this book also originated from the time when Professor K. van 't Riet (then a Gist brocades employee) together with Professor J.J. Heijnen (then a Gist brocades employee, now with Delft Technical University) cotaught a course on biochemical engineering with Dr. N.W.F. Kossen (then at Delft Technical University, now with Gist brocades). A substantial portion of Part Three originates from this lecture series.

The authors wish to acknowledge Dr. N.W.F. Kossen and Prof. J.J. Heijnen for the contribution they made to this book. Prof. A. Prins is acknowledged for Chapter 12.

Further, a number of the examples were contributed by Dr. L.E.S. Brink, Ir. C.D. de Gooyer, Ir. M.H. Zwietering and Dr. P. Verlaan. Dr. R.G.J.M. van der Lans and Mr. B. Brandt made many useful suggestions and corrections.

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PART I

Introduction



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1

Introduction

1.1 Defining the subject

Bioreactor design is an integral part of biotechnology, an area with rather loose and contested borders. A widely distributed, much advertised, but unfortunately wrong definition of biotechnology is:

Microbiology
Genetics
Biochemistry
Engineering
Chemistry
Pharmacy
Food Technology

BIOTECHNOLOGY

Biotechnology is not simply the sum of microbiology, genetics, biochemistry, engineering, etc.; no, it is the integration of these disciplines, and this involves quite a bit more than just simple addition. Integration and application are the keywords which can be found in most definitions of biotechnology (Fig. 1.1). Especially when designing bioreactors, integration of biological and engineering principles is essential. The bioreactor should be designed such that specific biological and technological demands of a process are met. Naturally, quality and price of the product are decisive for commercial realization. The aim of bioreactor design can thus be defined as "minimization of the costs of the pertinent product while retaining the desired quality, and this within the biological and technological constraints." This does not mean à priori that minimizing the costs of the bioreactor also means minimizing the costs of the integral process. This depends largely on the cost-determining part(s) of the process. If running the bioreactor is cost determining, then maximization of the overall volumetric productivity of

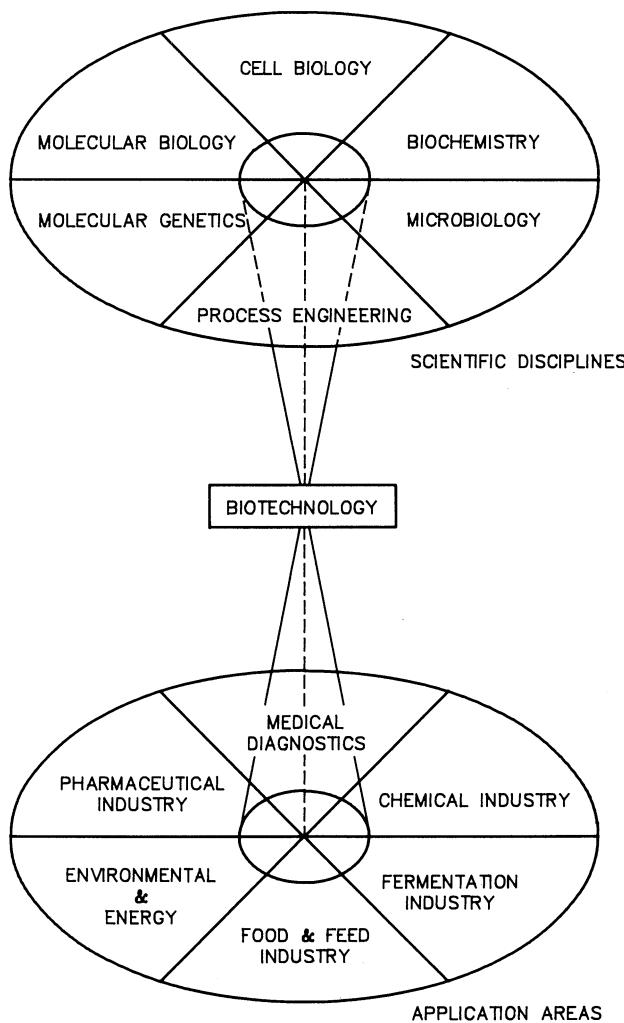


Fig. 1.1 Biotechnology: application-oriented integration of biodisciplines and engineering.

the bioreactor is, in general, the rational approach. If, on the other hand, the downstream processing is cost determining, then maximization of the product concentration in the bioreactor is, in general, the rational thing to do. However, here again integration is the keyword. Bioreactor design should be an integral part of the overall process design.

The words bioreactor, biocatalyst and product have been used in general terms. In the following sections of this chapter the bioreactor will be defined, though still in general terms, with respect to reactor concepts and types and to tools in bioreactor design. The meaning of product is obvious with the annotation that biomass can be the desired product too. In this book biocatalyst means either an enzyme, an enzyme complex, a cell organelle or a whole cell. The latter can be growing or nongrowing, viable or nonviable, etc. Furthermore, a biocatalyst can be free or immobilized, which has far-reaching consequences not only with respect to mass transfer, but sometimes also for the physiology of viable cells. Integration of mass transfer and biokinetics is essential in the description (microkinetics) of immobilized biocatalysts. The source of biocatalysts can be of either microbial, plant or animal origin and examples of all three are used in this book.

1.2 Productivity and product concentration

1.2.1 Overall volumetric productivity

Overall volumetric productivity Q_p ($\text{mol m}^{-3} \text{ s}^{-1}$) (it is also common to use a yearly basis) is the average production capacity per unit volume and time of the bioreactor. The overall volumetric productivity is confined on the one hand by physical constraints such as mass and heat transfer, and on the other hand by biocatalyst concentration C_x (mol m^{-3}) and activity of the biocatalyst, expressed as substrate consumption rate $-r_s^u$ ($\text{mol m}^{-3} \text{ s}^{-1}$). Maximization of the overall volumetric productivity of the bioreactor in principle means minimization of the costs of investment, because one can suffice with smaller equipment. It usually also means lower operating costs. In general, it means too that it is desired to operate the bioreactor as close as possible to the physical constraints, the horizontal dotted line in Fig. 1.2. This physical limitation is the result of mass and heat transfer limitations, which are stoichiometrically related to product formation. The vertical dotted line in Fig. 1.2 symbolizes the limitation which is a consequence of the fact that the concentration of the biocatalyst is bound to certain defined limits, for instance solubility in case of isolated enzymes and "space" in case of suspended cells. Fig. 1.2 also shows that the biocatalyst should have a minimum specific activity to be able to operate the bioreactor close to its physical ceiling.

1.2.2 Overall biocatalyst productivity

In addition to limitations by mass and heat transfer and concentration of biocatalyst, the overall volumetric productivity of the bioreactor is deter-

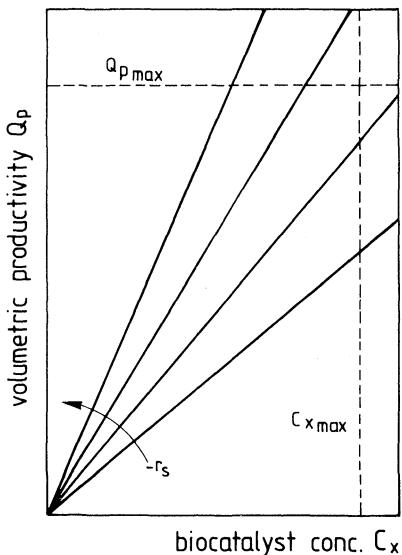


Fig. 1.2 Constraints of overall volumetric productivity. (Adapted from Cooney, 1983.)

mined by the overall productivity of the biocatalyst, Pr_{px} (-), defined as the total moles of product which are produced by 1 mol of biocatalyst during its operational lifetime t_l (s). Pr_{px} is related to the specific product production rate q_p (s^{-1}) (moles of product produced per mol of biocatalyst per second) as

$$Pr_{px} = \int_0^{t_l} q_p dt = - \int_0^{t_l} Y_{ps}^{ov} \frac{r_s^u}{C_x} dt \quad (1.1)$$

The definition of Y_{ps}^{ov} (-), the overall yield of product on substrate (total moles of product produced per total mol of substrate), leads to

$$Q_p = \frac{1}{t_l} \int_0^{t_l} q_p C_x dt \quad (\text{mol m}^{-3} \text{s}^{-1}) \quad 1.2a$$

$$Q_p = \frac{1}{t_l} \int_0^{t_l} -Y_{ps}^{ov} r_s^u dt \quad (\text{mol m}^{-3} \text{s}^{-1}) \quad 1.2b$$

The time needed to empty, clean, refill, restart, etc., the bioreactor between two operations is the so-called down-time, which is symbolized by t_d (s). In case t_d is relevant it can be introduced in Eq. (1.2) by replacing $1/t_i$, preceding the integral, by $1/(t_i + t_d)$. In addition to the molar productivity used above, the mass productivity (kg product instead of mol) is also quite commonly used in engineering (conversion from one to the other by means of the molecular weights). It is also common practice to use hour, day or year as unit of time.

The search for and the development of a useful biocatalyst with a suitable yield, specific activity and stability is in the beginning the task of microbiologists, biochemists, molecular biologists, protein engineers, etc. However, especially with respect to stability, the process engineer also has means available, among others immobilization, to improve the stability of biocatalysts. This is covered in this book.

1.2.3 Product concentration

The effect of the composition of the product stream leaving the bioreactor on the costs of the downstream processing is large. Therefore, it is essential to take this into account when designing the bioreactor. This often means in practice that the bioreactor is designed such that the concentration of product is as high as possible. The end concentration of product C_p (mol m⁻³) in the bioreactor depends on r_s^u , Y_{ps}^{ov} and the residence time in the bioreactor. For a batch reactor, with t_b (s) as the time that the batch lasts, this leads to

$$C_p = Y_{ps}^{ov} \int_0^{t_b} -r_s^u dt \quad (\text{mol m}^{-3}) \quad 1.3$$

and for a continuous reactor with a liquid throughflow F_l (m³ s⁻¹) and a volume V (m³):

$$C_p = -Y_{ps}^{ov} r_s^u \frac{V}{F_l} \quad (\text{mol m}^{-3}) \quad 1.4$$

Concentration of product is especially a key-parameter when the downstream processing is the cost-determining part of the integral process. Product recovery is often a laborious and expensive operation, especially when diluted aqueous solutions are involved such as we usually encounter in biotechnology. However, it has become clear that the aqueous reaction medium, which was for a long time supposed to be essential for biocatalysis, can be replaced to a large extent by a suitable organic solvent (Laane et al., 1987).

1.3 Bioreactor types

1.3.1 The stirred vessel

In Fig. 1.3 a schematic view of a stirred vessel is given. The vessel is cylindrical with a height H_v (m) and a diameter T_v (m). Usually H_v is equal to or greater than $2 T_v$. It is equipped with a stirrer in the lower compartment. This stirrer is mounted near the bottom usually at a distance equal to the stirrer diameter. At a lower position the stirrer and bottom interact leading to a decrease in power consumption. At a higher position liquid circulation problems can occur because at increased gas flow rate in case of aeration the bubbles will not be recirculated in the lower compartment. Sometimes the upper compartment(s) are also equipped with a stirrer. The vessel is equipped with baffles to prevent rotation of the contents as a whole. For aeration an air sparger is mounted below the stirrer. For mass transfer its construction is generally not relevant, so it is chosen on the basis of sterility and cleaning criteria.

Fig. 1.4 shows a number of stirrers that are used. It will be shown in Chapter 11 that the stirrer is needed to provide a certain level of power input needed

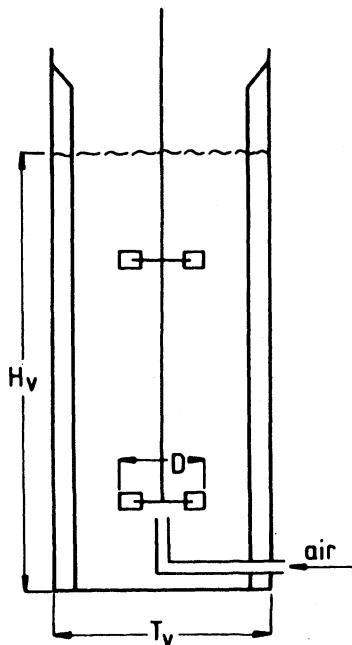


Fig. 1.3 Schematic representation of a stirred fermenter.

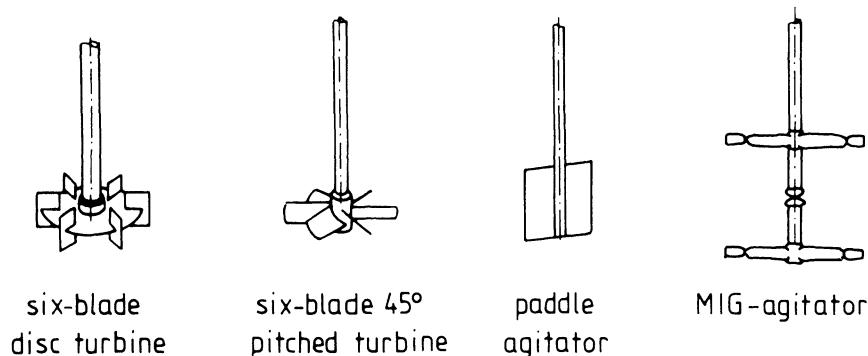


Fig. 1.4 Schematic representation of a number of stirrers. (Adapted from Zlokarnik, 1972.)

for aeration purposes. Therefore, the turbine stirrer, being easy to construct and having a high power number, is the most widely used. The other types are less intensively applied. A detailed description of all types of stirrers can be found in Zlokarnik (1972).

Special design considerations like stirrer drives and sealings are not dealt with here. Also self-aerating stirrers are not discussed. Information about them can be found in Sittig (1983).

1.3.2 The bubble column

A schematic representation of this simple reactor is given in Fig. 1.5. Usually it is $H_v \geq 2 T_v$. At the bottom a sparger is mounted. To prevent too heterogeneous flow patterns in the lower compartment, the sparger nozzles have to be distributed over the cross section of the bottom. Therefore, one ring or a small number of parallel pipes or a starlike construction of pipes is commonly used. In the pipes holes are drilled. In the chapter on mass transfer it is shown that complicated spargers or very small holes merely have disadvantages for most applications.

1.3.3 The air lift

The air lift consists of two pipes, interconnected at top and bottom. In one of the pipes (the riser) air is sparged at the bottom. The air rises and escapes at the top. Therefore, under most circumstances there is no air present in the other pipe (the downcomer). The density difference between riser and

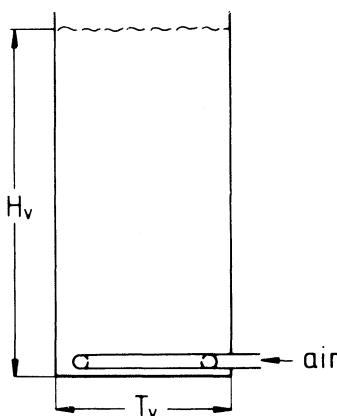


Fig. 1.5 Schematic representation of a bubble column reactor.

downcomer causes an intensive liquid circulation. Two designs can be used, i.e., the internal (Fig. 1.6A) and the external loop reactor (Fig. 1.6B). When an internal loop reactor is built underground, we refer to this as a deep shaft. Volumes can be up to thousands of m^3 . H_v generally is much larger than T_v , usually of the order of $10 T_v$, but for the deep shaft up to $100 T_v$.

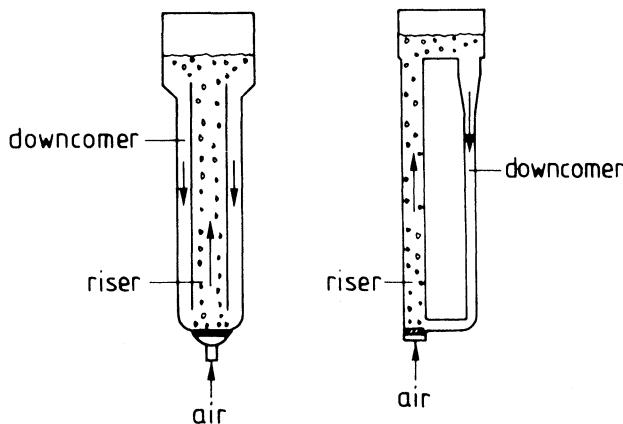


Fig. 1.6 Schematic representation of the air lift. A. Internal loop reactor. B. External loop reactor.

1.3.4 The packed bed

The packed bed is very simple in nature and differs largely from the other three types. It consists of a tubular pipe, packed with the biocatalyst particles. It can be operated in the upflow or downflow mode, i.e., the reaction medium is introduced either at the bottom or the top of the reactor.

1.3.5 Discussion of reactor types

The four basic reactor types discussed in this book are the ones described above. The difference between the packed bed and the other three is so great that the choice usually is straightforward. On the basis of detailed mixing, mass transfer and cost calculations a rational choice can be made between stirred vessel, bubble column and air lift. Many times, however, this is not needed and the following general rules suffice.

- Mixing and mass transfer do not differ very much between the three types. In the air lift the flow is controlled, which enables the introduction of a controlled substrate addition at more than one place.
- For mass transfer the maximum attainable value is higher for stirred vessels, because of the larger power that can be introduced with the stirrer. For air lift and bubble column mass transfer collapses above a viscosity of about 0.1 N s m^{-2} .
- At increasing scale mechanical problems can occur in the stirred vessel because of the large power values of the stirrer motor.

Based on these three reasons the following rules of thumb apply:

- In those cases where viscosities can rise above 0.1 N s m^{-2} (mycelial, biopolymer fermentations) a stirred vessel is chosen because air lift and bubble column will fail.
- In those cases where flexibility in viscosity and mass transfer is needed (pilot plant) a stirred vessel is chosen because air lift and bubble column cannot offer this flexibility.
- In low viscosity fermentations at large scale ($50\text{--}500 \text{ m}^3$) a bubble column is chosen because it is the cheapest fermenter.
- In low viscosity fermentations at very large scale ($200\text{--}10,000 \text{ m}^3$) an air lift is chosen because it permits local and controlled substrate addition. (The stirred fermenter would offer immense mechanical problems at $V > 500 \text{ m}^3$ because the stirrer power P_s could easily rise above 1 MW).
- Viscous fermentations cannot be scaled up to scales $> \approx 500 \text{ m}^3$ because the stirred fermenters that are needed for these cases will offer mechanical problems ($P_s > 1 \text{ MW}$).

These rules of thumb explain why most fermenters $< 200 \text{ m}^3$ are stirred (viscous, pilot) or bubble type (SCP, Single Cell Protein) while the very large ones are of the more recently developed air lift type.

1.4 Reactor concepts

1.4.1 Introduction

The bioreactor has been introduced in general terms in Section 1.3. In this section the basic bioreactor concepts, i.e., the batch, the fed-batch, the continuous-flow stirred-tank reactor (CSTR), the cascade of CSTRs and the plug-flow reactor, will be described. Integration with the (micro)kinetics, in other words the kinetics of the pertinent free biocatalysts or of the immobilized biocatalysts including mass transfer, yields the overall reactor description or macrokinetics in later chapters. In order to come up with these descriptions, a mass balance over the bioreactor should be drawn up (Fig. 1.7).

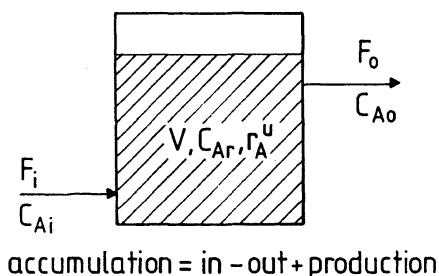
In words:

The accumulation of a compound A with a concentration in the reactor C_{Ar} is equal to the amount of A that comes in, subtracted by the amount that goes out, and augmented by the amount that is produced.

In formula:

$$\frac{d(V C_{Ar})}{dt} = F_i C_{Ai} - F_o C_{Ao} + \int r_A^u dV \quad (\text{mol s}^{-1}) \quad 1.5$$

In this equation V is the liquid volume in the bioreactor (m^3), C_A the concentration of A (mol m^{-3}) by which the subscripts i and o refer to



accumulation = in - out + production

Fig. 1.7 The mass balance over the bioreactor.

the concentration in the influent and effluent, respectively, t is time (s), F_i and F_o the flow ($\text{m}^3 \text{s}^{-1}$) of the in and outgoing stream, respectively, and r_A^u the production rate per unit volume ($\text{mol m}^{-3} \text{s}^{-1}$) of A .

1.4.2 The batch reactor

The most-prominent characteristic of the batch reactor is the fact that there are no in- and outgoing flows (Fig. 1.8). This means that all that is produced is accumulated. The mass balance [Eq. (1.5)] thus simplifies to, assuming ideal mixing:

$$\frac{d(V C_{Ar})}{dt} = r_A^u V \quad (\text{mol s}^{-1}) \quad 1.6$$

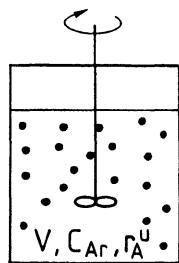
When the volume V remains constant, Eq. (1.6) even further simplifies:

$$\frac{dC_{Ar}}{dt} = r_A^u \quad (\text{mol m}^{-3} \text{s}^{-1}) \quad 1.7$$

With boundary conditions $C_{Ar} = C_{Ar}(0)$ on $t = 0$ and $C_{Ar} = C_{Ar}(t_b)$ at $t = t_b$, the time one batch lasts, separation of variables and integration lead to:

$$t_b = \int_{C_{Ar}(0)}^{C_{Ar}(t_b)} \frac{dC_{Ar}}{r_A^u} \quad (\text{s}) \quad 1.8$$

Substitution of the pertinent rate equation yields the time a run should last to obtain a desired conversion.



accumulation = production

Fig. 1.8 The batch reactor.

1.4.3 The fed-batch reactor

The distinguishing feature of the fed-batch reactor is that there is only an ingoing flow and no outgoing flow (Fig. 1.9). Eq. (1.5) thus becomes, assuming ideal mixing:

$$\frac{d(V C_{Ar})}{dt} = F_i C_{Ai} + r_A^u V \quad (\text{mol s}^{-1}) \quad 1.9$$

or

$$V \frac{d(C_{Ar})}{dt} + C_{Ar} \frac{dV}{dt} = F_i C_{Ai} + r_A^u V \quad (\text{mol s}^{-1}) \quad 1.10$$

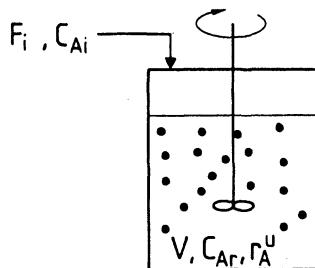
This equation cannot be solved analytically without further simplification and data.

1.4.4 The continuous-flow, stirred-tank reactor (CSTR)

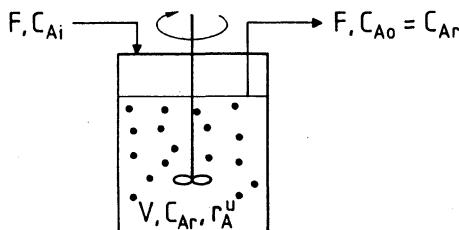
The CSTR defined here (Fig. 1.10) is that the volume V is constant and ideally mixed and that the inflow equals the outflow, i.e. $F_i = F_o = F$. For solving the mass balance [Eq. (1.5)] of a CSTR it is assumed that the reactor essentially is in a steady state:

$$\frac{d(V C_{Ar})}{dt} = 0 \quad (\text{mol s}^{-1}) \quad 1.11$$

Eq. (1.5) thus becomes



accumulation = in + production Fig. 1.9 The fed-batch reactor.



$$0 = \text{in-out} + \text{production}$$

Fig. 1.10 The CSTR.

$$0 = F C_{A_i} - F C_{A_o} + r_A^u V \quad (\text{mol s}^{-1}) \quad 1.12$$

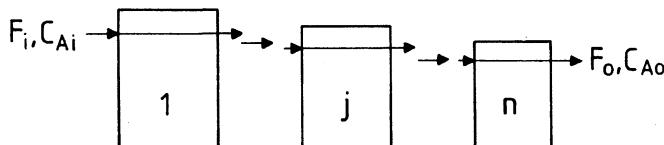
or

$$\tau_{CSTR} = \frac{V}{F} = \frac{C_{A_o} - C_{A_i}}{r_A^u} \quad (\text{s}) \quad 1.13$$

in which τ_{CSTR} is the average residence time (s). As in an ideally mixed vessel the concentration in the reactor is equal to C_{A_o} , this equation can immediately be solved by substituting for r_A^u the appropriate rate equation with $C_{A_r} = C_{A_o}$.

For n CSTRs in series (Fig. 1.11) the same assumptions are made for each vessel as for the single CSTR above. For each vessel in the series Eq. (1.13) thus holds:

$$\tau_{CSTR(j)} = \frac{V_j}{F} = \frac{C_{A_r(j)} - C_{A_r(j-1)}}{r_A^u} \quad (\text{s}) \quad 1.14$$



$$0 = \text{in-out} + \text{production}$$

Fig. 1.11 The cascade of CSTRs.

The subscript j refers to the j -th vessel. In later chapters the optimal design for fermentation using the Monod equation with a linear relation between the microorganism and substrate concentration will be worked out, as well as the optimal design for n CSTRs in series containing (immobilized) biocatalyst of constant activity and following Michaelis-Menten kinetics.

1.4.5 The plug-flow reactor

In the ideal plug-flow reactor (Fig. 1.12) the continuous phase flows as a plug through the reactor; i.e., there is no mixing or, in other words, no axial dispersion. Consequently, if a compound is consumed or produced, a concentration gradient will exist in the direction of flow. The mass balance is therefore first set up over an infinite small slice perpendicular to the direction of the flow with volume dV of the bioreactor. Assuming steady state and $F_i = F_o = F$, Eq. (1.5) then is reduced to:

$$0 = F C_{Ar} - F(C_{Ar} + dC_{Ar}) + r_A^u dV \quad (\text{mol s}^{-1}) \quad 1.15$$

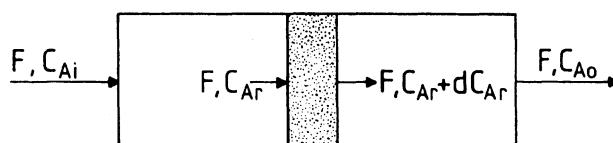
Rewriting gives:

$$\frac{dC_{Ar}}{r_A^u} = \frac{dV}{F} \quad (\text{s}) \quad 1.16$$

Integration over the whole reactor:

$$\int_{C_{Ai}}^{C_{Ao}} \frac{dC_{Ar}}{r_A^u} = \int_0^V \frac{dV}{F} \quad (\text{s}) \quad 1.17$$

or:



$$0 = \text{in-out} + \text{production}$$

Fig. 1.12 The plug-flow reactor.

$$\tau_{pf} = \frac{V}{F} = \int_{C_{Ai}}^{C_{Ao}} \frac{dC_{Ar}}{r_A^u} \quad (s) \quad 1.18$$

in which τ_{pf} (s) is the residence time of the plug-flow reactor. This equation is in principle the same as that of the batch reactor and integration with the (micro)kinetics is identical.

1.4.6 Discussion of the reactor concepts

A comparison of the various types of reactor concepts, in a general sense, is actually only possible between the batch, the CSTR and the plug-flow reactor. The cascade of CSTRs, depending on the number of vessels n in the series, more or less behaves as an ideal mixer for $n \rightarrow 1$ or an ideal plug flow for $n \rightarrow \infty$. The fed-batch reactor is more difficult to situate. Although the concentration of compounds important for the rate of reaction can be controlled optimally during the whole fed period, the reactor volume is only partially utilized, especially in the beginning. Nevertheless, this reactor concept certainly has decisive advantages in many cases as shown by the fact that it is one of the most widely used.

For the batch, the CSTR and the plug-flow reactor the following equations for the "residence time" have been derived, respectively:

$$t_b = \int_{C_{Ar}(0)}^{C_{Ar}(t_b)} \frac{dC_{Ar}}{r_A^u} \quad (s) \quad 1.8$$

$$\tau_{CSTR} = \frac{C_{Ao} - C_{Ai}}{r_A^u} \quad (s) \quad 1.13$$

$$\tau_{pf} = \int_{C_{Ai}}^{C_{Ao}} \frac{dC_{Ar}}{r_A^u} \quad (s) \quad 1.18$$

In general, the rate of reaction decreases if the reactant concentration decreases (reaction-order > 0). For a CSTR this means that the rate of reaction is low for the whole reactor as it is determined by the low concentration in the reactor, equal to that of the outflow. For the other two

reactor types the conversion takes place at concentrations less than the higher incoming concentration. This means that in case of "ordinary" kinetics $\tau_{CSTR} > t_b = \tau_{pf}$, in other words that the CSTR should be larger than the batch and the plug-flow reactor in order to accomplish the same degree of conversion. Naturally, the down-time of the batch reactor is not taken into account here.

In an autocatalytic reaction, i.e., the more product the faster the rate of reaction, or when there is substrate inhibition, the $1/r_A^u$ versus C_A , curves can look quite different. In that case $t_b = \tau_{pf} > \tau_{CSTR}$. For these types of kinetics it is thus advantageous to use a CSTR. Only when zero-order kinetics are involved is there no difference in "residence times" and thus volumina, of the three types of reactors.

1.5 Overall outline

As can be extracted from the preceding sections, the overall volumetric productivity of the bioreactor forms the basis of this book. Built on that are chapters concerning general aspects such as reactor concepts and types, balances, product concentration and medium (viscosity, coalescence, etc.). On the same level are chapters dealing with the limitations of the biocatalyst, i.e., yield, kinetics and stability, as well as chapters discussing the physical constraints resulting from mass and heat transfer, mixing, foaming, flooding, hold-up, shear and power consumption. Integration of kinetics and mass transfer on the particle level in immobilized biocatalysts yields the microkinetics. Similarly, the effect of diffusion limitation of substrate on the apparent stability of the immobilized biocatalyst can be quantified. Integration of (micro)kinetics in the various reactor concepts yields the macrokinetics. These last chapters form the integration block. In this block a division between gas/liquid and liquid/solid mass transfer is carried out for reasons of clarity. Taking into account all pertinent parameters derived in the various preceding blocks, the overall reactor models for the batch, the fed-batch and the continuous stirred-tank reactor (CSTR), the cascade of CSTRs and the plug-flow reactor are derived. Finally, the process engineering aspects and the economic evaluation form the last part. The various chapters are illustrated by means of exercises.

1.6 Examples

Example 1.1 The productivity of an enzyme reaction

Glucose is isomerised into fructose by means of immobilized glucose isomerase in a continuous bioreactor.

Calculate :

- The overall productivity of the biocatalyst
- The overall productivity of the bioreactor on a yearly basis
- The product concentration at time $t = 0$ and $t = t_l$

Data:

- $-\frac{r_s^u(T)}{C_x} = 10^{12} e^{-7.5 \times 10^4 / RT} \text{ s}^{-1}$
- $r_s^u(t) = r_s^u(0) e^{-k_d t} \text{ kg m}^{-3} \text{ s}^{-1}$
- $k_d = 10^{35} e^{-2.5 \times 10^5 / RT} \text{ s}^{-1}$
- $Y_{ps} = 0.9 \text{ kg kg}^{-1}$
- operational life time = half-time $t_{0.5}$ of biocatalyst (s)
- concentration of biocatalyst C_x is 0.1 kg m^{-3}
- $F = 0.01 \text{ m}^3 \text{ s}^{-1}$
- $V = 100 \text{ m}^3$
- $T = 305, 310, 315 \text{ and } 320 \text{ K}$
- down-time is 1 week
- $R = \text{gas constant} = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$

(Kinetic data adapted from Roels, 1983)

Solution:

$$r_s^u(t_l) = r_s^u(t_{0.5}) = \frac{1}{2} r_s^u(0) = r_s^u(0) e^{-k_d t_l}$$

Rewriting: $t_l = t_{0.5} = \frac{\ln 2}{k_d}$

Substitution in Eq. (1.1) gives

$$Pr_{px}(t) = -Y_{ps} \int_0^{t_l} \frac{r_s^u(0)}{C_x} e^{-k_d t} dt$$

or

$$Pr_{px}(t_{0.5}) = -Y_{ps} \frac{r_s^u(0)}{C_x} \frac{(1 - e^{-k_d t_l})}{k_d} = -\frac{Y_{ps} r_s^u(0)}{2 k_d C_x}$$

Introducing the temperature dependency yields subsequently

$$Pr_{px}(t_{0.5}, T) = \frac{Y_{ps} 10^{12} e^{-7.5 \times 10^4 / RT}}{2 \times 10^{35} e^{-2.5 \times 10^5 / RT}}$$

and for the overall volumetric productivity on a yearly basis

$$Q_p = \frac{365}{t_{0.5} + 7} C_x Pr_{px}(t_{0.5}, T)$$

The product concentration in this case can be described by

$$C_p = \frac{Y_{ps} r_s^u(t, T) V}{F}$$

Substituting the given data in these equations gives Table 1.1

It is clear that as result of the down-time an optimum occurs and obvious that at $t = t_{0.5}$ the concentration of product is half of that at $t = 0$.

Example 1.2 Production of D-(-)-4-hydroxyphenylglycin

D-(-)-4-hydroxyphenylglycin is the optically active intermediate in the synthesis of the broad-spectrum antibiotic amoxicilline. This intermediate is among others produced from a hydantoin derivative by means of the enzyme hydantoinase. The hydantoin derivative is poorly soluble in water,

Table 1.1 The productivity of glucose isomerase

| T (K) | 305 | 310 | 315 | 320 |
|--------------------|--------------------|--------------------|--------------------|--------------------|
| $r_s^u(0)$ | 0.14 | 0.23 | 0.36 | 0.56 |
| $Pr_{px}(t_{0.5})$ | 4.4×10^6 | 1.4×10^6 | 0.5×10^6 | 0.2×10^6 |
| $t_{0.5}$ (days) | 552 | 112 | 24 | 5.4 |
| Q_p | 2.85×10^5 | 4.38×10^5 | 5.72×10^5 | 5.04×10^5 |
| $C_p(0)$ | 127 | 204 | 324 | 507 |
| $C_p(t_{0.5})$ | 63 | 102 | 162 | 254 |

about 1 kg m^{-3} . The price of the substrate is cost determining and the degree of conversion should therefore be very high, at least 99%.

Calculate the volume needed to produce 1000 kg of product per day by immobilized hydantoinase in a

- batch reactor
- CSTR
- plug-flow reactor

Data:

- Michaelis-Menten reaction kinetics with $v_{max} = 1.5 \times 10^{-4} \text{ kg s}^{-1} \text{ m}^{-3}$ biocatalyst and $K_m = 5 \times 10^{-3} \text{ kg m}^{-3}$
- $Y_{ps} = 1 \text{ kg kg}^{-1}$
- degree of conversion 99%
- down-time for batch reactor 1 day
- The activity of the biocatalyst can be assumed to be constant in time.
- The reactors contain 0.1 m^3 immobilized biocatalyst per m^3 , except the plug-flow reactor, which is packed with 0.5 m^3 immobilized biocatalyst per m^3 reactor.

Solution for the batch reactor:

$$t_b = \int_{C_{Ar}(0)}^{C_{Ar}(t_b)} \frac{dC_{Ar}}{Y_{ps} r_A^u} = - \int_{C_{sr}(0)}^{C_{sr}(t_b)} \left(\frac{0.1 v_{max} C_{sr}}{K_m + C_{sr}} \right)^{-1} dC_{sr}$$

$$t_b = \frac{1}{0.1 v_{max}} \left[K_m \ln \left(\frac{C_{sr}(0)}{C_{sr}(t_b)} \right) + (C_{sr}(0) - C_{sr}(t_b)) \right]$$

Substituting the numerical values gives:

$$t_b = 67535 \text{ s} = 18.76 \text{ h.}$$

Taking into account the down-time of about 1 day, it can be concluded that 1 batch takes about 2 days. The concentration of substrate at time zero is 1 kg m^{-3} and the yield 1 kg kg^{-1} . Therefore, to produce 1000 kg d^{-1} , one batch should have the size of 2000 m^3 , which is thus the working volume of the batch reactor. Note that $0.1 v_{max}$ is substituted due to the biocatalyst loading of $0.1 \text{ m}^3 \text{ m}^{-3}$.

Solution for the CSTR:

$$\tau_{CSTR} = \frac{V}{F} = \frac{C_{A0} - C_{Ai}}{r_A^u} = \frac{(C_{si} - C_{so})(K_m + C_{so})}{0.1 v_{max} C_{so}}$$

Substituting the numerical values yields

$$\tau_{CSTR} = 99 \times 10^3 \text{ s} = 27.5 \text{ h.}$$

In order to produce 1000 kg d⁻¹ the flow rate should be:

$$F = \frac{1000 \text{ kg d}^{-1}}{(1 \text{ kg m}^{-3})(24 \text{ h d}^{-1})} = 41.7 \text{ m}^3 \text{ h}^{-1}$$

$$V = \tau_{CSTR} \times F = 1146 \text{ m}^3$$

The minimum working volume of the CSTR is thus 1146 m³.

Solution for the plug-flow reactor

$$\begin{aligned} \tau_{PFR} &= \int_{C_{si}}^{C_{so}} \frac{dC_{Ar}}{r_A^u} = - \int_{C_{si}}^{C_{so}} \frac{0.5v_{max} C_{sr}}{K_m + C_{sr}} dC_{sr} \\ \tau_{PFR} &= \frac{1}{0.5v_{max}} \left[K_m \ln\left(\frac{C_{sr}}{C_{so}}\right) - (C_{so} - C_{si}) \right] \end{aligned}$$

Substituting the numerical values gives

$$\tau_{PFR} = 13507 \text{ s} = 3.75 \text{ h.}$$

$$V = \tau_{PFR} \times F = 3.75 \times 41.7 = 156 \text{ m}^3.$$

As result of the continuous character of the plug-flow reactor (negligible down-time) and the high volumetric activity, the required volume for the plug-flow reactor is relatively low.

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PART II

Basics



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2

Balances

2.1 Introduction

A standard tool in process engineering is the use of elemental balances. According to this method, the balances are written down for each separate chemical element. For bioreactor design, they provide useful information about the flows through and production in the apparatus. For instance, at a given sugar feed rate to a fermenter, growth and maintenance of the microorganisms determine the ammonia consumption rate. Because accumulation should be prevented, the ammonia feed rate to the fermenter should have the same value. Balances can provide the relation between sugar and ammonia feed rate. In the same way the total mass of the fermenter and the concentrations at the end of the fermentation can be calculated. These data can be used to calculate the fermenter volume that is required and can be used as input variables for downstream processing design. In the design of a new production plant balances are the basis for the whole flow sheet. Finally, it should be possible to provide design criteria based on very limited input data such as: "This amount of *BOD* should be removed," or "That amount of product should be produced in a day." This chapter will deal with the principles of balances for bioreactor design.

2.2 Elemental balances

2.2.1 General principles

The principle behind the elemental balances is very simple: The elemental flow into the system equals the flow out of the system, corrected for accumulation in the system. The production term is absent in this balance equation because elements are neither formed nor destroyed.

$$F'_{Ai} - F'_{Ao} = \frac{dVC_A}{dt} \quad (\text{mol s}^{-1}) \quad 2.1a$$

in which

| | | |
|-----------|--|------------------------|
| F'_{Ai} | = flow rate of element A entering the system | (mol s ⁻¹) |
| F'_{A0} | = flow rate of element A leaving the system | (mol s ⁻¹) |
| V | = volume (of the system) | (m ³) |
| C_A | = concentration of element A (in the system) | (mol m ⁻³) |
| t | = time | (s) |

If accumulation is absent Eq. (2.1a) leads to:

$$F'_{Ai} = F'_{A0} \quad (\text{mol s}^{-1}) \quad 2.1b$$

In Eq. (2.1a) and (2.1b) the kinetics or any other indication about conversion processes of the molecules that contain the elements is absent. It is a black box or unstructured model. The equations can be written down for all elements that are relevant. The elements flow in and out of the system as part of the flows, like the substrate flow, in and out of the system. Fig. 2.1 shows the flow rates that are relevant for a given fermentation with a given N, S, P and C substrate. The system is not defined as the fermenter, but as the biomass only. It is also defined in such a way that accumulation of any of the components is absent. This allows it to replace the flow rates by the production rates r . The following remarks can be made:

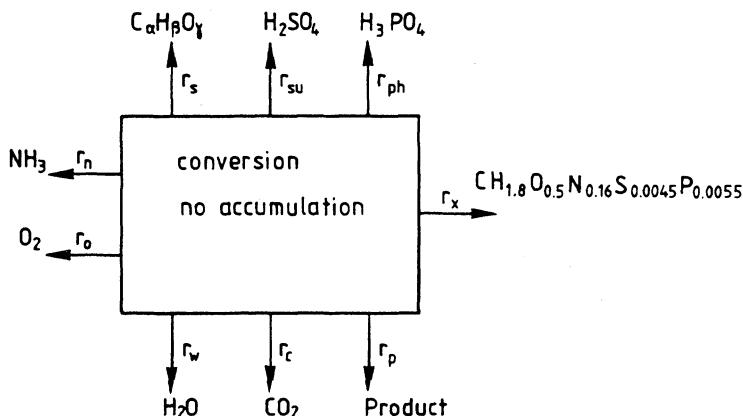


Fig. 2.1 The production rates in the black box model.

- All rates are regarded as production rates. This means that a consumption like that of the C substrate (r_s) appears in the equations as a production with a negative value. Where rates are mentioned as consumption this is given as the production rate preceded by a negative sign. This also explains that all arrows in Fig. 2.1 have to be directed out of the system. It may be noted that there does not exist a consistent sign convention for the rates. Therefore care should be taken in using literature data.
- The black box system consists of only the biomass and the substrates and products processed by the biomass. Because this usually will not be identical to the fermenter contents, the production rates do not always have the same value as the feed rates to the fermenter, because the production rates are only that part that is taken out or added to the fermenter contents by the biomass. For instance, for a batch fermentation the production rates are equal to the amounts produced (or consumed from the available substrates in the batch) by the microorganism.
- Accumulation in the black box model is assumed to be absent. This can be done because the system boundary is defined as the microorganisms only. It is assumed that accumulation of a given compound within the microorganism does not occur. The system boundary definition states that in case accumulation of feed components in the fermenter occurs, this accumulation is kept outside of the model. Product and biomass accumulation is generally included in the model, by a stepwise procedure. For biomass growth the inclusion in the black box is needed because the newly formed biomass will participate in the conversion processes. At a defined t value these accumulations for a time dt are calculated from the production rates. Then these values are added to the mass present in the model at $t = t$, to give the mass at $t = t + dt$.
- Substrates can vary from fermentation to fermentation. In Fig. 2.1 only the C substrate is given with a variable composition. The N, P and S substrates are given with a fixed composition, to prevent the equations to be derived from becoming too complicated. If a substrate different from those given here is used, the equations can easily be set up again, following the lines given in this chapter.
- To determine the elemental balances the elemental composition of all flows, i.e., production rates, has to be known. For biomass this is complicated. However, usually biomass composition is rather constant. It has been determined experimentally (reviewed by Roels, 1983) that for most applications the composition given in Fig. 2.1 can be used. Normalized to C_1 basis it corresponds to a molar mass of 24.4 g.

By application of Eq. (2.1b) the elemental balances are made by careful addition of the contribution of all production rates for each element. For example, for carbon the production rate of 1 mol of C substrate involves α atoms of carbon, that of biomass involves 1 atom and that of CO_2 also involves 1 atom. In the other production rates no carbon is involved. In this way Eq. (2.2a) is formed. In the same way Eqs. (2.2b-2.2f) are obtained as the balance equations for the other elements. These equations are derived for use when accumulation is absent and $r_p = 0$.

Eq. (2.2) shows the balances for the elements given in the first column, derived from the production rates of the molecules given in the upper row:

| | $\text{CH}_{1.8}\text{O}_{0.5}..$ | $\text{C}_\alpha\text{H}_\beta\text{O}_\gamma$ | NH_3 | O_2 | CO_2 | H_2O | H_2SO_4 | H_3PO_4 | |
|------------|-----------------------------------|--|---------------|--------------|-----------------------|----------------------|-------------------------|-------------------------|------------------|
| Carbon | $1r_x$ | | $+\alpha r_s$ | | $+1r_c$ | | | | $= 0 \quad 2.2a$ |
| Hydrogen | $1.8r_x$ | | $+\beta r_s$ | $+3r_n$ | | $+2r_w$ | $+2r_{su}$ | $+3r_{ph}$ | $= 0 \quad 2.2b$ |
| Oxygen | $0.5r_x$ | | $+\gamma r_s$ | | $+2r_o + 2r_c + 1r_w$ | $+4r_{su}$ | $+4r_{ph}$ | | $= 0 \quad 2.2c$ |
| Nitrogen | $0.16r_x$ | | | $+1r_n$ | | | | | $= 0 \quad 2.2d$ |
| Sulfur | $0.0045r_x$ | | | | | $+1r_{su}$ | | | $= 0 \quad 2.2e$ |
| Phosphorus | $0.0055r_x$ | | | | | | $+1r_{ph}$ | | $= 0 \quad 2.2f$ |

It can also be written in matrix notation. With Φ as the matrix containing the production rate row

$$\Phi = (r_x \ r_s \ r_n \ r_o \ r_c \ r_w \ r_{su} \ r_{ph}) \quad (\text{mol s}^{-1}) \quad 2.3$$

and \mathbf{E} as the matrix for the elemental flow columns

$$\mathbf{E} = \left(\begin{array}{ccccccc} 1 & 1.8 & 0.5 & 0.16 & 0.0045 & 0.0055 \\ \alpha & \beta & \gamma & 0 & 0 & 0 \\ 0 & 3 & 0 & 1 & 0 & 0 \\ 0 & 0 & 2 & 0 & 0 & 0 \\ 1 & 0 & 2 & 0 & 0 & 0 \\ 0 & 2 & 1 & 0 & 0 & 0 \\ 0 & 2 & 4 & 0 & 1 & 0 \\ 0 & 3 & 4 & 0 & 0 & 1 \end{array} \right) \quad (-) \quad 2.4$$

The balances become

$$\Phi \cdot \mathbf{E} = 0 \quad (\text{mol s}^{-1}) \quad 2.5$$

This matrix notation can be very useful for computer calculations, particularly when substrates of different composition have to be included.

2.2.2 The elemental balances

The 6 balance equations given above contain 8 variables; thus all rates can be written as a function of 2 of the other rates. When we are mainly interested in biomass growth and if carbon is the main substrate, r_x and r_s are chosen as the parameters. The equations can then be rewritten as:

$$r_c = -1 r_x - \alpha r_s \quad (\text{mol s}^{-1}) \quad 2.6a$$

$$r_o = +1.094 r_x + (\alpha + \beta/4 - \gamma/2) r_s \quad (\text{mol s}^{-1}) \quad 2.6b$$

$$r_w = -0.647 r_x + (-\beta/2) r_s \quad (\text{mol s}^{-1}) \quad 2.6c$$

$$r_n = -0.16 r_x \quad (\text{mol s}^{-1}) \quad 2.6d$$

$$r_{su} = -0.0045 r_x \quad (\text{mol s}^{-1}) \quad 2.6e$$

$$r_{ph} = -0.0055 r_x \quad (\text{mol s}^{-1}) \quad 2.6f$$

These equations are derived without any knowledge about the reactions that take place in the reactor.

Without further knowledge about these reactions it is not possible to simplify these relations further. Biochemical reactions are extremely complicated and modeling of the complete reaction scheme is impossible. However, only knowledge about the relation between the rates given in Eq. (2.6) is needed. In Chapter 3 it will be shown that generally the substrate consumption rate shows a linear relation with the biomass production rate and the biomass M_x (mol):

$$-r_s = \frac{r_x}{Y_{xs}} + m_s M_x \quad (\text{mol s}^{-1}) \quad 2.7$$

The yield value Y_{xs} (-) and the maintenance coefficient m_s (mol substrate per mol biomass per second) both appear to be nearly constant if conditions do not change too drastically. Eq. (2.7) shows that r_x and r_s cannot be chosen independently and after that be introduced in Eq. (2.6). They are related to each other by the biomass present, the yield value and the maintenance coefficient. The first term on the right-hand side of Eq. (2.7) gives the amount of substrate needed for growth and the second term the

amount needed for maintenance. At a given substrate flow rate, the maintenance requirements always have to be fulfilled and the amount of substrate given by the second term is used for this process. Then the growth rate is given by the amount of substrate that remains after subtracting the maintenance requirements. By introducing the linear growth equation, our black box model is transformed into a gray box. The equations then can be given as a function of a single rate value and the biomass. The last one can be regarded as a starting value at time zero. Eq. (2.6) gives, when r_s is chosen as the single variable:

$$r_x = -Y_{xs} r_s - m_s Y_{xs} M_x \text{ (mol s}^{-1}\text{)} \quad 2.8a$$

$$r_c = (-\alpha + Y_{xs}) r_s + m_s Y_{xs} M_x \text{ (mol s}^{-1}\text{)} \quad 2.8b$$

$$r_o = (\alpha + \beta/4 - \gamma/2 - 1.094 Y_{xs}) r_s - 1.094 m_s Y_{xs} M_x \text{ (mol s}^{-1}\text{)} \quad 2.8c$$

$$r_w = (-\beta/2 + 0.647 Y_{xs}) r_s + 0.647 m_s Y_{xs} M_x \text{ (mol s}^{-1}\text{)} \quad 2.8d$$

$$r_n = 0.16 Y_{xs} r_s + 0.16 m_s Y_{xs} M_x \text{ (mol s}^{-1}\text{)} \quad 2.8e$$

$$r_{su} = 0.0045 Y_{xs} r_s + 0.0045 m_s Y_{xs} M_x \text{ (mol s}^{-1}\text{)} \quad 2.8f$$

$$r_{ph} = 0.0055 Y_{xs} r_s + 0.0055 m_s Y_{xs} M_x \text{ (mol s}^{-1}\text{)} \quad 2.8g$$

Some remarks are appropriate here:

- Eq. (2.8) is the generally valid general balance equation [Eq. (2.6)] combined with a model for substrate consumption [Eq. (2.7)] and therefore not anymore generally valid.
- r_s is used here as a parameter because in continuous culture and fed batch this rate can be used as a control variable. Of course, other parameters can be chosen like r_o if oxygen is the limiting substrate or r_x if the growth rate needs to be controlled, with r_x related to the specific growth rate μ (s^{-1}) by

$$r_x = \mu M_x \text{ (mol s}^{-1}\text{)} \quad 2.9$$

- As parameter usually the rate is chosen that can be controlled in the process or the rate that has to follow a predefined pattern during the fermentation.
- Some insight can be gained by qualitative interpretation of the equations. Eq. (2.8a) shows that the growth rate becomes zero when the substrate feed rate equals the maintenance requirements. Eq. (2.8c) gives insight into the oxygen requirements depending on growth. The equation shows that oxygen is required at any r_s value because both terms on

the right-hand side have a negative value (r_s has a negative value). The last part on the right-hand side of the equation is the maintenance-related oxygen consumption. At a given r_s , maintenance always will lead to an extra oxygen consumption because both terms are negative.

- For nitrogen, sulfate and phosphorus both a substrate and maintenance term appear. However, they are of opposite sign when the actual values are introduced. The substrate flow rates define the maximum rates for N, S and P when all substrate would be used for growth. Maintenance diminishes the amount of substrate available for growth and therefore the rate of N, S and P because they are needed only for cell growth. By combining Eqs. (2.8e-f) with Eq. (2.8a) it can be seen that at zero growth the rates for N, S and P indeed become zero.
- To solve the seven Eqs. (2.8) with the nine unknown parameters, two parameter values have to be known. Usually this will include the starting value of the amount of biomass at time zero. The other parameter can be a control variable like one of the substrate rate patterns or the growth rate pattern. The way to use the equations is shown in Examples 2.1 and 2.2.

2.3 The heat-balance equations

A balance can also be set up for the free enthalpies of the flows entering and leaving the system. The difference between these quantities is the heat production r_H (W) of the system. An extensive discussion is presented by Roels (1983). Here we will only discuss the main features.

Enthalpy has to be defined relative to a reference level. Usually enthalpy of combustion to CO₂, (liquid) H₂O and N₂ is chosen because these compounds are not transformed further by the microorganisms. With ΔH (W) defined as the free enthalpy of combustion of component i , the heat balance reads

$$r_s \Delta H_s + r_x \Delta H_x + r_n \Delta H_n + r_p \Delta H_p + r_H = 0 \quad (\text{W}) \quad 2.10$$

Phosphorus and sulfate have been left out of the equation. Their contribution is less than 1%. This balance can be solved for each specific case.

For aerobic fermentations it can be shown that there is a simpler way to calculate heat production values. Roels (1983) and other authors have shown that for aerobic production the heat production can be related to the oxygen consumption, because the consumption of oxygen delivers 460×10^3 J mol⁻¹, within very reasonable margins.

$$r_H = -460 \times 10^3 r_o \quad (\text{aerobic}) \quad (W) \quad 2.11$$

This equation is rather accurate for a wide range of conditions, also for most fermentations in which a product is made. Examples 2.3 and 2.4 show how heat production values can be calculated.

For anaerobic conditions such a general rule cannot be derived. Here the enthalpy difference between product and substrate is the main determining factor. For engineering purposes it is preferred in anaerobic fermentations to relate the heat production to the C-substrate consumption rate. This is worked out in Example 2.4.

2.4 The total mass balance

For a commercial fermentation, particularly when executed as a fed batch, the total weight balance of the system is important. This can be set up by adding all feed rates. The water that is usually part of the substrate flows also should be added, as well as the vapor in the outflowing air. The balance differs from the balance given in the preceding chapters because it is a balance for feed rates, not for production rates. Also, the system boundary is now the fermenter instead of the microorganisms. Example 2.5 shows such a balance.

2.5 Conclusions

The balances can be set up for each fermentation. They provide, when combined with the kinetics, the data needed for engineering purposes. They also are a useful tool in that they give the data for the different feed streams. Also, the heat production and the weight development of the fermenter can be calculated.

2.6 Examples

Example 2.1 The effects of carbon and oxygen limitation

Usually the mass of inoculation, $M_x(0)$, in a fermenter has a low value. Thus in the first period of the fermentation the growth is limited by the maximum growth rate of the microorganism. This is called the exponential growth phase. Exponential growth automatically leads to a limitation of one of the substrates after a period of time. Here we will simulate the examples of carbon and oxygen limitation.

- Carbon limitation

Assume that the carbon feed rate is limited by the value $r_{s,max}$. For the exponential growth phase where the carbon is not yet limiting the following calculation can be made.

After rewriting Eq. (2.9) into

$$r_x = \frac{dM_x}{dt} = \mu_{max} M_x \quad (\text{mol s}^{-1}) \quad 2.12$$

this can be solved as

$$M_x = M_x(0) e^{\mu_{max} t} \quad (\text{mol}) \quad 2.13$$

Using Eq. (2.7) leads to the substrate consumption rate:

$$r_s = -\left(\frac{\mu_{max}}{Y_{xs}} + m_s\right) M_x(0) e^{\mu_{max} t} \quad (\text{mol s}^{-1}) \quad 2.14$$

Eqs. (2.13) and (2.14) can be used to calculate the other rates from Eqs. (2.8b-2.8g).

At time $t = t'$, at the value of M'_x , r_s just will reach the value $r_{s,max}$. For $t \geq t'$ we then deal with limitation in carbon substrate feeding rate, i.e.,

$$r_s = r_{s,max} \quad (\text{mol s}^{-1}) \quad 2.15$$

For $t \geq t'$ it can also be derived by introduction of the first part of Eq. (2.12) into Eq. (2.8a) and followed by integration

$$M_x = \left(M'_x + \frac{r_{s,max}}{m_s}\right) e^{Y_{xs}m_s(t'-t)} + \frac{-r_{s,max}}{m_s} \quad (\text{mol}) \quad 2.16$$

Eqs. (2.13) and (2.16) can be used to calculate all the rates at all times, as is illustrated in Fig. 2.2, which was generated using the following data:

Fermenter volume 100 m³

C substrate C₁H₂O₁

$$\begin{aligned}
 r_{s, \max} &= -1.85 \text{ mol s}^{-1} \quad (= -2 \text{ kg glucose m}^{-3} \text{ h}^{-1}) \\
 \mu_{\max} &= 1.4 \times 10^{-4} \text{ s}^{-1} \quad (= 0.5 \text{ h}^{-1}) \\
 Y_{xs} &= 0.67 \text{ mol biomass (mol C}_1\text{H}_2\text{O}_1)^{-1} \quad (= 0.55 \text{ kg kg}^{-1}) \\
 m_s &= 4.6 \times 10^{-6} \text{ mol C}_1\text{H}_2\text{O}_1 \text{ (mol biomass)}^{-1} \text{ s}^{-1} \\
 &\quad (= 0.02 \text{ kg kg}^{-1} \text{ h}^{-1}) \\
 M_x(0) &= 4000 \text{ mol} \quad (= 1 \text{ kg m}^{-3}, \text{ with the mol mass of biomass} \\
 &\quad = 24.4 \text{ g})
 \end{aligned}$$

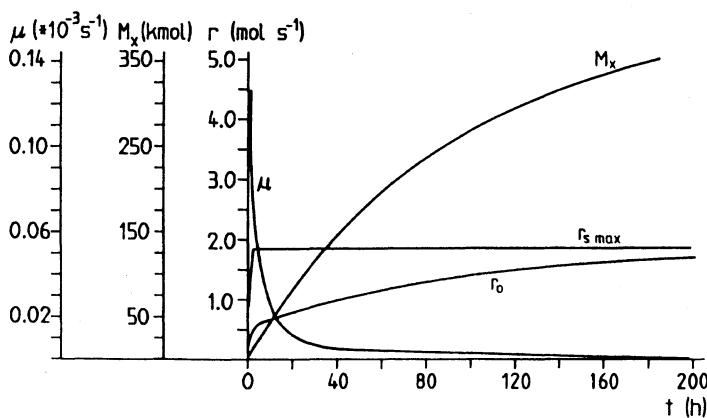
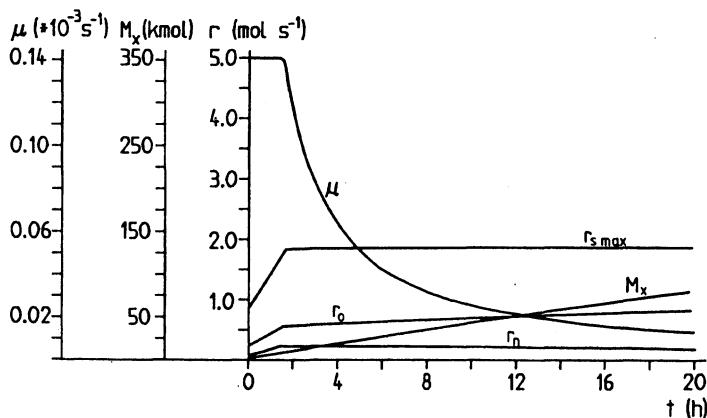


Fig. 2.2 Simulations for CH₂O limitation.

Fig. 2.2 shows an exponential growth phase up to $t = 1.5$ h. For $t > 1.5$ h, r_s limits the growth. The further increasing biomass leads to an increase of the relative part of the substrate that is used for maintenance. Because for a given amount of C substrate, maintenance consumes more oxygen than growth, the value of r_o slowly increases in time. Maintenance does not require any nitrogen, and therefore, r_n slowly decreases in time at $t > t'$. At maximum growth, maintenance requirements can be neglected.

$$\frac{\mu}{Y_{xs}} \gg m_s \quad (\text{s}^{-1}) \quad 2.17$$

as can be seen from combining Eq. (2.7) and Eq. (2.9) into

$$-r_s = \frac{\mu}{Y_{xs}} M_x + m_s M_x = \left(\frac{\mu}{Y_{xs}} + m_s \right) M_x \quad (\text{mol s}^{-1}) \quad 2.18$$

With the data used this means for this example that maintenance can be neglected at $\mu \gg 0.01 \text{ h}^{-1}$.

- Oxygen limitation

The equations can be derived in the same way as was done for the case of carbon limitation. The resulting simulations are given in Fig. 2.3, using as additional data: $r_{o,max} = -1.4 \text{ mol s}^{-1}$ ($OUR = 50 \text{ mol m}^{-3} \text{ h}^{-1}$). In the exponential growth phase the phenomena are the same as for the preceding case. When there is oxygen limitation, however, the increase of biomass with time leads to an increasing part of the oxygen being used for maintenance. For maintenance, less carbon source is needed for a given amount of oxygen used than for growth, and therefore, the carbon consumption rate decreases with time. Finally, a value will be reached at which all carbon and oxygen are needed for maintenance. In that case the growth becomes zero.

Example 2.2 The influence of the degree of oxidation

The degree to which the substrate is oxidized before consumption is important when oxygen limitation occurs. Fig. 2.4 presents simulations for $\text{C}_1\text{H}_2\text{O}_1$, C_1H_4 and $\text{C}_1\text{H}_4\text{O}_1$ using the same data as given in Example 2.1. Fig. 2.4 shows the tremendous effect of the oxidation state of the substrate. The differences in the biomass that can be produced can differ by more than a factor of 2.

Example 2.3 The heat production

The heat production in an aerobic fermentation is calculated for the case of r_s limitation presented in Example 2.1. Both Eqs. (2.10) and (2.11) are

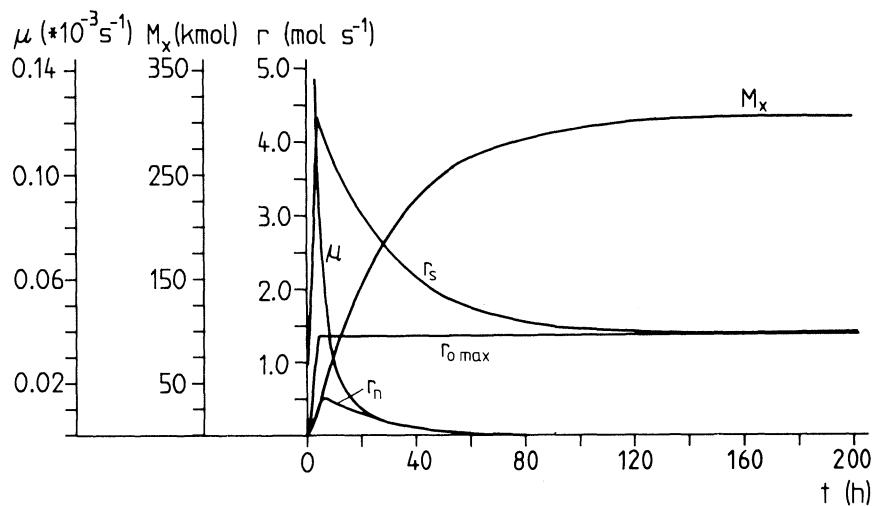
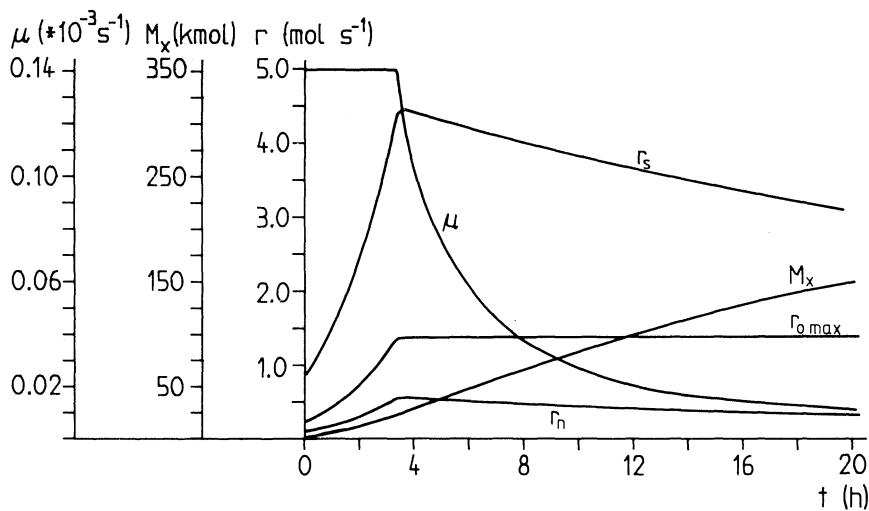


Fig. 2.3 Simulations for oxygen limitation.

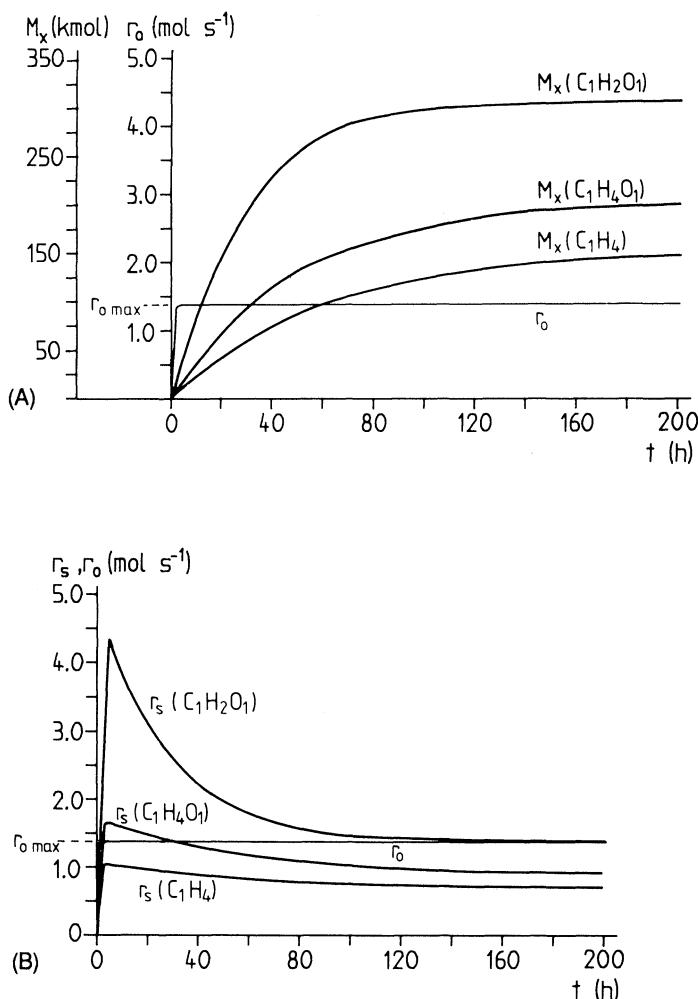


Fig. 2.4 The influence of the substrate degree of oxidation.

used. The enthalpy of combustion data is taken from Roels and is based on gaseous CO_2 and N_2 and liquid H_2O .

Data used: Same as in Example 2.1

$$\Delta H_s = 479 \times 10^3 \text{ J} (\text{mol C}_1\text{H}_2\text{O}_1)^{-1}$$

$$\Delta H_x = 541 \times 10^3 \text{ J} (\text{mol C}_1\text{H}_{1.8}\text{O}_{0.5}\text{N}_{0.16})^{-1}$$

$$\Delta H_n = 329 \times 10^3 \text{ J} (\text{mol N}_1\text{H}_3)^{-1}$$

No product formation

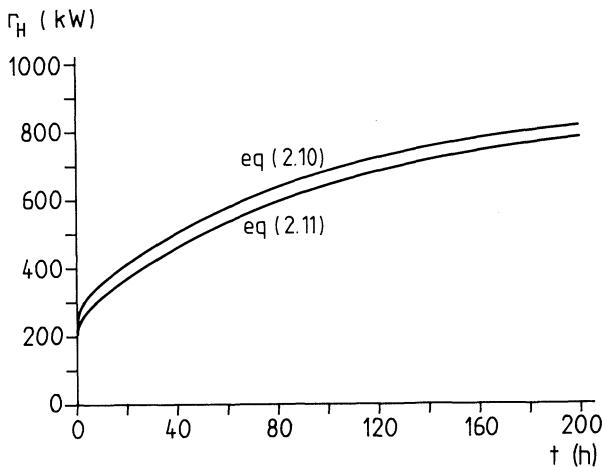


Fig. 2.5 The calculated heat production values.

The results given in Fig. 2.5 show that Eqs. (2.10) and (2.11) both predict rather similar heat production values. Small deviations always occur, because ΔH is dependent on a large number of parameters, such as molarity, pH and temperature. In Fig. 2.2 it can be seen that r_o increases with fermentation time. Eq. (2.11) then predicts and Fig. 2.5 confirms that this results in an increase of the heat production. We have already stated that at constant r_s , this is caused by the decrease in growth rate during fermentation. When less oxidized substrates like CH_4 are used, the heat production will be considerably higher than for CH_2O because the oxygen consumption is considerably higher.

Example 2.4 The heat production of aerobic and anaerobic fermentations

The heat production related to substrate consumption rate for aerobic and anaerobic fermentations is calculated in this example.

- Aerobic

For an aerobic fermentation without product formation Eq. (2.10) together with the ΔH data of Example 2.3 leads to: