Basic Bioreactor Design

Klaas van 't Riet
Johannes Tramper

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Johannes Tramper

Wageningen Agricultural University
Wageningen, The Netherlands

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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 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. Kossen and Prof. Heijnen for the contribution they made to this book.
Further, a number of the examples were contributed by Dr. L. Brink, Ir. C. de Gooyer, Ir. M. Zwietering and Dr. P. Verlaan.
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PART I
Introduction

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

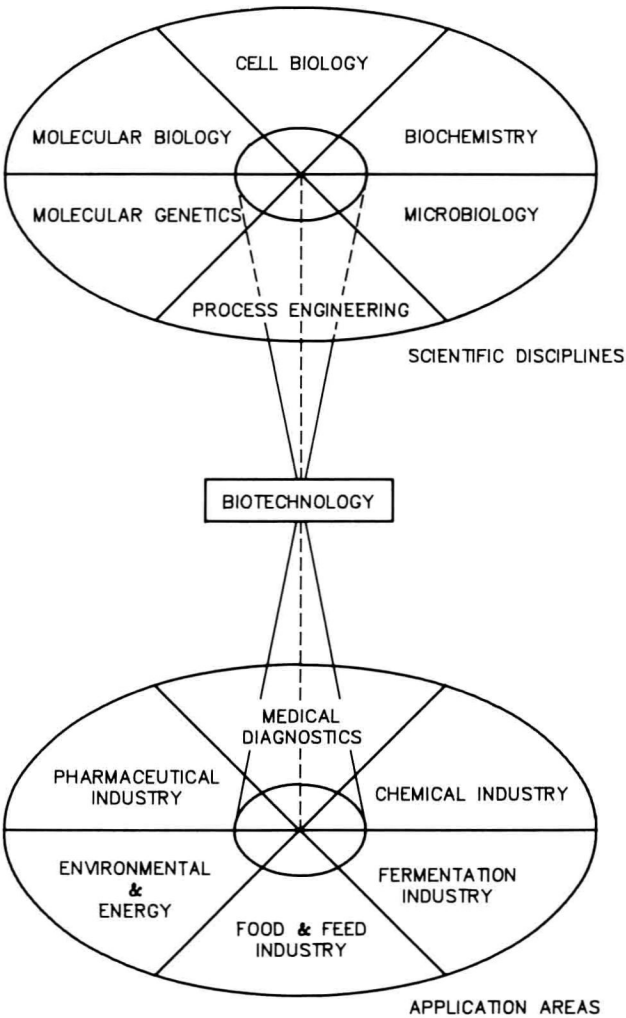

Fig. 1.1 Biotechnology: application-oriented integration of biodisciplines and engineering.

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 (mol m^{-3} 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 $-r_s''$ (mol m^{-3} 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-

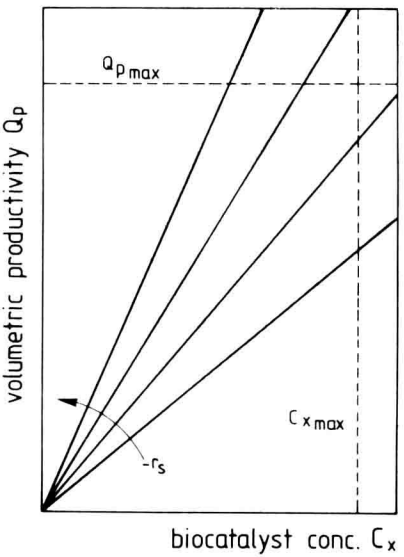

Fig. 1.2 Constraints of overall volumetric productivity. (Adapted from Cooney, 1983.)

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

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

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

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

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

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 $1/t_l$, preceding the integral, by $1/(t_l+t_d)$. 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 great. Therefore, it is essential to take this into account when designing the bioractor. 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 is proportional to r_s^u, C_x, Y_{ps}^{ou} 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

$$C_p = -Y_{ps}^{ou} r_s^u t_b \qquad \text{(mol m}^{-3}\text{)} \qquad 1.3$$

and for a continuous reactor with a liquid throughflow F_l (m^3 s^{-1}) and a volume V (m^3):

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

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 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

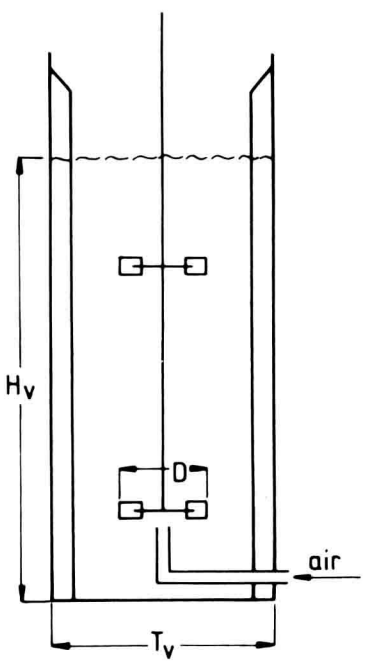

Fig. 1.3 Schematic representation of a stirred fermenter.

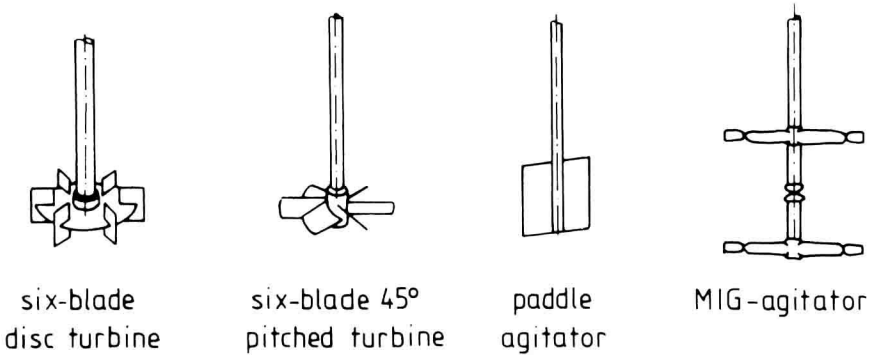

Fig. 1.4 Schematic representation of a number of stirrers. (Adapted from Zlokarnik, 1972.)

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

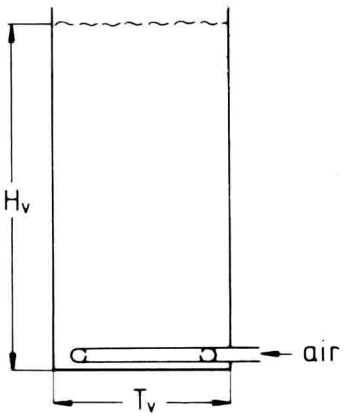

Fig. 1.5 Schematic representation of a bubble column reactor.

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.

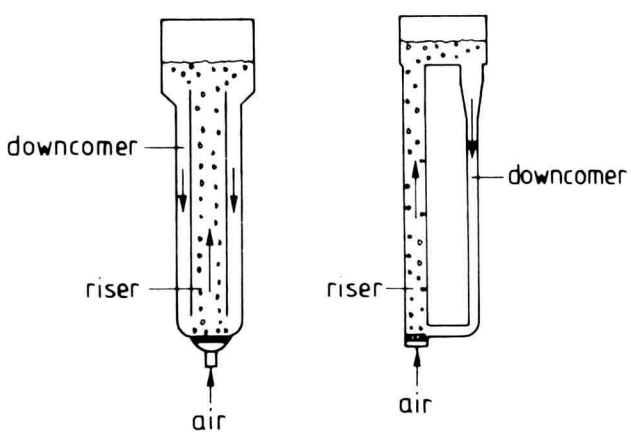

Fig. 1.6 Schematic representation of the air lift. A. Internal loop reactor. B. External loop reactor.

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 (50-500 m^3) a bubble column is chosen because it is the cheapest fermenter.
- In low viscosity fermentations at very large scale (200-10,000 m^3) an air lift is chosen because it permits local and controlled substrate addition. (The stirred fermenter would offer immense mechanical problems at $V > 500$ m^3 because the stirrer power P_s could easily rise above 1 MeW.
- Viscous fermentations cannot be scaled up to scales $> \approx 500$ m^3 because the stirred fermenters that are needed for these cases will offer mechanical problems ($P_s > 1$ MeW).

These rules of thumb explain why most fermenters < 200 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, substracted by the amount that goes out, and augmented by the amount that is produced.

In formula:

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

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

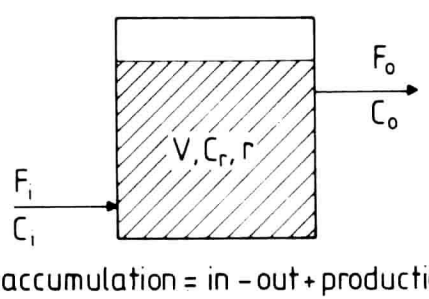

Fig. 1.7 The mass balance over the bioreactor.