BIOREACTION ENGINEERING

Reactions Involving
Microorganisms and Cells

VOLUME 1

Fundamentals, Thermodynamics, Formal Kinetics, Idealized Reactor Types and Operation Modes

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Fundamentals, Thermodynamics, Formal kinetics, Idealized reactor types and Operation modes

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Bioreaction engineering
Volume 1

Fundamentals, Thermodynamics, Formal kinetics, Idealized reactor types and Operation modes

The original German edition of this book is a volume in the

Principles of chemical technology series

Process technology for the chemical and related industries

Foreword to German edition

Scarcely any other discipline has become so involved with other scientific fields to such great effect as chemical engineering has over the last few decades. The vast oil and gas deposits discovered during the post-war period started off the process and more recently, the concern over natural resources, energy supplies and environmental issues has helped to keep it going. Biologists, geologists and medical experts are all aware of the theoretical and practical importance of process engineering and chemical engineers incorporate the latest developments from the fields of mathematics, information technology, physics and electrotechnology amongst others into their work.

Although this mutual involvement is to be welcomed, it does make literature reviews rather difficult and this is where the 'Principles of Chemical Technology' series comes in. As stated in the foreword to the first series, it covers the principles of physics, chemistry, measurement and control technology, materials and process technology as used in basic physical processes and chemical reaction technology.

The publishers are aware that it is impossible to complete the overall concept by extending the series into the field of systems technology. All that can be done is to fit various pieces of the puzzle together depending on how topical a subject is and the level of expert knowledge available. Most specialists in a position to make a positive contribution are too heavily involved in their research and teaching, but there are always some who are prepared to find the time to give an overall summary and review of this enormously large field and even to write a book on the subject. Therefore, the publishers are confident that this series will produce work of benefit to a wide range of engineers, chemists and physicists working in the fields of process and machine technology both during their time as students and afterwards throughout their industrial career. At the same time, it is intended to provide a basis for new developments and openings into areas as yet unknown. The publishers welcome any support or constructive comments which could further the above objectives.

The series has also sparked off interest in other countries, several volumes being already translated into Spanish, French, English and Polish.

Author's Preface to German edition

Over the last few years, several books which give a good review of the biochemical engineering field have appeared: S. Aiba, A. E. Humphrey and N. F. Mills, *Biochemical Engineering*, Academic Press 1973 (second edition); S. J. Pirt, *Principles of Microbe and Cell Cultivation*, Blackwell Scientific Publications, 1975; J. E. Bailey and D. F. Ollis, *Biochemical Engineering Fundamentals*, McGraw-Hill Co. 1977; D. I. C. Wang, C. L. Cooney, A. L. Demain, P. Dunnill, A. E. Humphrey and M. D. Lilly, *Fermentation and Enzyme Technology*, Wiley Interscience 1979; the kinetics of cell growth and regulation are described in *Growth, Function and Regulation in Bacterial Cells*, by A. C. R. Dean and C. N. Hinshelwood, Oxford Univ. Press, London, 1966. Industrial microbiology is represented by H. J. Rehm's well-known book, *Industrielle Mikrobiologie*, Springer-Verlag, 1980 (second edition) and Prescott and Dunn's *Industrial Microbiology*, 4th edition, the Avi Publ. Co. 1982, editor G. Reed and these lay special emphasis on industrial application.

However, none of these books have bioreaction engineering as the focal point, hence the publishers of the 'Principles of Chemical Technology' series felt that there was a need for a book featuring the reaction engineering of cells and microorganisms as the central theme.

The aim of this book is to give an overall view of the current level of advance in bioreaction engineering and thereby assist those involved in this subject both at university and in industry. It is especially designed to appeal to young chemical engineers and process engineers and will, hopefully, stimulate their interest in this direction.

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1 Introduction

Fermentation is a long-established process which has expanded over the years to become the basis of biotechnology and biochemical engineering. It has experienced a renaissance during recent decades in the form of new fields such as mass production of secondary metabolites (e.g. antibiotics), biotransformation of organic substances such as steroids by microorganisms or cell cultures, biological treatment of effluent, sludge or animal waste as well as mass cultivation of microorganisms (e.g. for the production of enzymes for laundry detergents or of proteins as a foodstuffs and fodder additive) and the marked impact of genetic engineering.

Increase in demand combined with economic considerations have resulted in a steady increase in plant size, making the financial risk through faulty reactor construction increasingly greater.

Bioreaction engineering was established as a direct result of the need to reduce this risk by improving both constructional and operational aspects of the plant and can be divided up into two main aspects:

- macrokinetics of biological reactions;
- biological reactor engineering (bioreactor engineering)

The first aspect, known generally as macrokinetics of bioreactions, for short, deals with the interaction of biological, chemical and physical processes, such as cell growth, product formation, transport processes and surface phenomena taking place in bioreactors.

The kinetics of cell growth and product formation as well as simple structural models for describing dynamic cell behaviour form an essential basis on which macrokinetic assessment of bioreactions is made.

Hence, the principles of bioreaction macrokinetics cannot be established without close collaboration between biologists, microbiologists, process chemists and engineers.

Bioreactor engineering is mostly concerned with the reactor itself, with special emphasis on the influence of various types of reactors on biological, chemical and physical processes and vice versa. Chemical reaction models may also be used, provided that the special properties of biological media, e.g. change in medium rheology during cultivation by change in cell morphology, are also taken into consideration.

Once again, problems associated with bioreactor engineering can only be solved by close collaboration between the various specialist disciplines, biologists, microbiologists, process chemists and engineers being at the forefront.

Bioreactor construction, optimization and control are determined on the basis of both biological reaction macrokinetics and bioreactor engineering

aspects. The complex interaction between cell regulation, cell morphology, cell environment, reactor properties, process operation and reactor control (Fig. A) is another vitally important factor.

A system can only be closed-loop controlled if it is observable and controllable. Hence, facilities must be available so that

- cell behaviour,
- cell environment,
- cell morphology and
- reactor state

can all be observed and controlled. On-line measurement of cell behaviour is the most difficult aspect. Initial assessments are possible and the amount of ATP, NADH, DNA, RNA, protein and some enzyme activity can be measured on-line or quasi on-line, but methods of determination and their interpretation must be improved if they are to be used for control purposes [1.1–1.4]. On-line measurement of cell environment is not yet fully perfected either. The concentration of the major components dissolved in the medium can be measured on-line [1.5] but cell-mass concentration can not. Cell morphology can be indirectly determined by such as on-line measurement of the rheological behaviour [1.6, 1.7]. On-line measurement of reactor properties is not very advanced. There are a few publications on on-line measurement of local turbulence, scale of turbulence, power spectra, energy dissipation spectra and multiphase flow properties during cell cultivation [1.8, 1.9] but these can not be carried out as a matter of routine. A no-contact method of measurement such as laser Doppler anemometry or ultrasound would be of great value.

The system as shown in Fig. 1 could be controlled by setting up suitable dynamic (structured) models of cell regulation and reactor state, as well as linking these with cell environment and cell morphology as a function of control variables and testing the complex model experimentally.

Although our knowledge of cell regulation and reactor state is still very incomplete and our methods of observation and structured models still in their infancy, this book attempts to give a review of state which will show how far the current level of research has progressed.

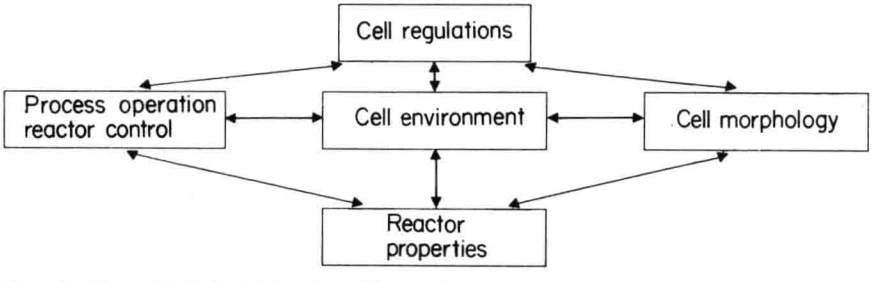

Fig. A. Complex interaction in a bioreactor

Volume I covers the foundations involved, Volume II the reactor properties and Volume III the complex interaction as depicted by Fig. A.

Volume I comprises the *modus operandi* of bioreactors, idealized reactor models and classical kinetics of cell growth and product formation as well as describing processes in idealized reactors, thus providing the fundamental concepts of bioreaction engineering.

Volume II describes actual submersed and surface reactors, compares them with each other and discusses selection and construction aspects. Measuring techniques characterizing bioreactors are also covered.

Volume III presents simple structured cell and reactor models, couples them together and simulates the dynamic behaviour of coupled systems, thus quantifying the complex interaction as shown in Fig. A. On-line methods for observing cell and reactor states are also described as is the processing of this information using the above models for the purpose of estimating bioreactor state and for its control.

Only reactors with microorganisms such as prokaryontae (bacteria) and eukaryontae (fungi yeast and moulds) as well as reactors with plant and animal cells are considered as part of the 'bioreaction engineering' series. Enzyme reactors are not included as there is no cell regulation, hence the complex interaction as shown in Fig. A is not present in the reactor.

Individual chapters are numbered in sequence throughout the three volumes and literature references follow suit.

Equations, figures and tables are numbered separately. However, when reference is made to an equation, figure or table in another volume, the volume number is given in roman figures, e.g. Vol. I.50 (Fig. 50 in Vol. I), Tab. II.10 (Table 10 in Vol. II), eqn. II.25 (eqn. 25 in Vol. II).

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2 Principles

Biological processes as they occur in nature and those which arise during industrial manufacturing procedures are extremely complex. Highly simplified mathematical models are used to describe them and an attempt is made to assess the difference between model and nature by using various correction factors, thus providing a more specific picture. This procedure establishes a kinetic pattern between cell growth and product formation.

The reactors used in industry are very different. They can be viewed from and characterized by various aspects. The kinetic theory of cell growth and product formation can be more easily described by combining the reactors into a few, highly simplified, idealized, basic groups which are easy to cope with mathematically. Differences between real and idealized reactors can be considered by means of additional parameters.

This mode of procedure is based on that used in the field of thermodynamics and reaction kinetics and adapts well to bioreaction engineering.

Reactors may be operated in various ways, the type of operation being a function of many parameters. Section 2.1 defines the most important types of operation, Section 2.2 deals with kinetic theory in idealized reactors and Section 2.3 discusses batch, continuous and semi-continuous operations.

2.1 MODUS OPERANDI OF REACTORS

Reactor operation may fall into any of the following categories:

1. discontinuous operation (batch process),
2. continuous and steady-state operation,
3. various types of semi-continuous operations such as extended culture or fed-batch process.

The **Batch process** is characterized by inoculation of the sterile culture medium with microorganisms, cultivated for a specific reaction period. During this time, cell, substrate (C-source, nutrient salts, vitamins etc.) and product concentrations alter. Good mixing ensures that there are no significant local differences in composition and temperature of the reaction mixture. The reaction is non-stationary.

As the oxygen in the culture medium is slightly soluble, a continuous oxygen supply is needed for aerobic cultivation, whilst removing the CO_2 formed in the same way. This is generally done by aeration of the medium. Hence, all batch reactors in which aerobic cultivation takes place are operated strictly on a semi-continuous basis. The gaseous products (e.g. CO_2) formed in batch-operated cultivations must be removed too. Hence, this type of plant also operates on a semi-continuous basis.

From now on, the expression 'batch operation' applies only to the liquid or

solid phase, so that particular aspects of the bioreaction process can be taken into account. The gas phase is left out of consideration.

Acid or alkali is added to the system periodically to control the pH value. Antifoaming agents by means of foam sensor are also added to the medium for chemical foam suppression. These medium corrections are ascribed to control procedures and also left out of consideration when defining process operation.

The *advantages* of batch operation are:

- lower investment costs as not much control is used,
- greater flexibility achieved by using a bioreactor for various products and product specification,
- higher conversion levels as a result of well-defined cultivation period,
- less risk of infection and cell mutation due to relatively short cultivation periods.

The *disadvantages* of batch operation are:

- non-productive idle time for filling, heating, sterilizing, cooling, emptying and cleaning the reactor,
- greater stress on measuring instruments due to frequent sterilization,
- higher expenditure due to preparing several subcultures for inoculum.
- high expenses as more personnel or process computers needed to control the non-stationary process (this can offset the advantage of lower investment costs),
- greater risk to service personnel from possible contact with some pathogenic microorganisms or toxic products.

Hence, discontinuous reactors are used when

- only small amounts of product are involved,
- one reactor is used to produce various products,
- there is a high risk of infection,
- there is a risk of microorganism mutation,
- when product separation from the cultivation medium is discontinuous.

Continuous operation is characterized in that the culture medium is fed continuously into the bioreactor — throughput must be constant if the steady state is involved. The medium added may be sterile or it may contain the microorganisms used. The reaction mixture is also drawn continuously from the reactor. All reaction variables and control parameters remain constant in time. As a result, a time-constant state is established in the reactor followed by constant productivity and output. The reaction is in steady state in this case.

The *advantages* of continuous operation are:

- large scope for mechanization and automation,
- low wages bill,
- lower reactor volume, as there are no unproductive idle periods taken up with emptying, filling and sterilization of the reactor,

- product quality is constant as operating conditions are invariant,
- less possible danger to service personnel from pathogenic microorganisms or toxic materials, due to improved mechanization,
- less wear and tear on instruments from sterilization.

The *disadvantages* of continuous operation are:

- low flexibility as only slight changes (throughput, medium composition, oxygen concentration, temperature) are possible,
- raw material quality must be uniform as operating conditions can not be adapted so easily,
- high investment costs, caused mainly by control and automation equipment and continuous sterilization of the medium,
- continuous renewal of non-soluble, solid substrates (e.g. straw) can be very expensive,
- high risk of infection due to long cultivation periods,
- high risk of microorganism mutation due to long cultivation periods.

In view of all the advantages and disadvantages mentioned above, continuous operation is preferred for processes with high production rates, for gas, liquid or soluble solid substrates and when microorganisms with high mutation stability are involved.

Semi-continuous operation can be regarded as a combination of batch and continuous operation.

Many variations of this type of process are practised. The most popular involves starting the reactor off as a batch process and when the growth-limiting substrate (generally C-source) has been consumed, it is either fed to the reactor in a specified manner (fed-batch), or the concentration of the substrate is kept constant by extended culture. Moreover, programmed substrate addition is frequently practised for secondary metabolite production, in which cell growth and product formation often occur in separate phases.

The *advantages* of semi-continuous operation are:

- high level of flexibility,
- semi-stationary method of operation even in the case of slightly mutating microorganisms and those involving an infection risk,
- high yield as a result of well-defined cultivation time (no cells are added or taken away during the cultivation period in semi-continuous operation. Hence the reactor operates batchwise as far as the microorganisms are concerned),
- environmental conditions of the microorganisms may be optimized to suit the growth phase or production phase and age of culture.

The *disadvantages* of semi-continuous operation are:

- non-productive idle time for filling, heating, sterilizing, cooling, emptying and cleaning the reactor,
- higher wages bill due to greater staffing or use of process computers.

Expensive instruments may also be required (e.g. to keep the substrate concentration constant),
- greater possible risk to service personnel of contact with pathogenic microorganisms or toxic products,
- more wear and tear on instruments from frequent sterilization.

Semi-continuous processes are often practised when continuous methods are not possible (e.g. due to slight mutation or infection of microorganisms) and batch production would result in low productivity figures. Like batch reactors, semi-continuous reactors are non-stationary.

2.2 IDEALIZED REACTOR TYPES

Most bioreactors used in practice can be described by mathematical models representing a combination or extension of a few basic idealized types of reactor as outlined below.

2.2.1 Basic Types

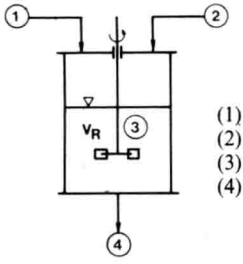
In the case of the **ideally mixed stirred batch reactor** (Fig. 1), intensive stirring ensures ideal backmixing, thus preventing any local differences in process variables such as temperature and concentration. However, these do in fact alter during the course of time as a function of cell growth, substrate consumption and product formation. Microorganisms are inoculated at time $t = 0$ after the reactor has been filled and the medium sterilized, mass concentrations being X^0, S^0 and P^0 at this stage. The change in process parameters as a function of time t depends on biological microorganism parameters and reaction conditions (see Section 2.3). Reaction is stopped at time t^e and the reactor emptied. Product concentration is characterized by cell mass concentration X^e. Substrate concentration S^e and product concentration p^e. Change in concentration of component i in the reactor can be described by the simple formula

$$\frac{dC_i}{dt} = R_i \tag{1}$$

in which R_i is the formation or consumption rate of component i
C_i is the concentration of i in the medium: $C_i (= X, S, P \ldots)$,
X is dry cell mass concentration,
S is substrate mass concentration,
P is product mass concentration,
$t = 0$, $C_i = C_i^0$ are the initial conditions.

Section 2.3 gives the various formulae for R_i.

In a **continuously operated ideally mixed stirred tank reactor** (Fig. 2), the sterile ($X_0 = 0$) or cell-enriched ($X_0 > 0$) medium is fed to the vessel, subjected to an intensive stirring process and then drawn off. V represents the medium



(1) Filling, Sterilization
(2) Injection ($t=0$)
(3) Bioreaction process
(4) Emptying, cleaning

When $t=0$ (Injection) $X=X^0,\ S=S^0,\ P=P^0$
$t=t^1$ (Cultivation) $X=X^1,\ S=S^1,\ P=P^1$
$t=t^e$ (End of Cultivation) $X=X^e,\ S=S^e,\ P=P^e$

(3) Bioreaction process

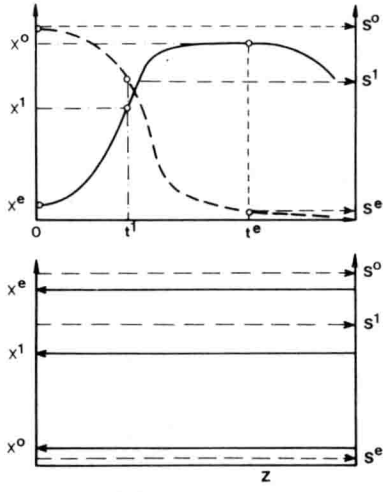


Fig. 1. Ideally mixed stirred tank batch reactor

throughput volume. X_0, S_0 and P_0 represent the mass concentrations in the feed product and X_e, S_e and P_e the concentrations in the final product. Process variables are regarded as uniform throughout the reactor, hence, the medium has the same composition both inside the reactor and in the exit stream. At the reactor inlet $Z=0$, the medium composition changes according to a step function from X_0, S_0 and P_0 to X_e, S_e and P_e, as the component mixing time is negligeable in comparison with the residence time inside the reactor.

The presence of an ideally mixed state in a continuously operated stirred reactor can be determined in various ways:

- the steady-state concentration of the reaction components is measured as a function of the local coordinates in the reactor: if concentration is independent of position, then presence of ideal backmixing is confirmed;
- by means of stimulus-response techniques, e.g. by changing the concen-