Process Simulation and Modeling, 1

Modeling and Optimization of Fermentation Processes

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PREFACE

The ancient alchemists stopped hammering at the iron rod in attempts to convert it into gold when solid scientific predictions taught them the impossibility of the task. Much too often, however, it seems that we are behaving in a similar way in our attempts to make the cell synthesize valuable products for us. To run a fermentation process is still more of an art than an exact science. We should not be fooled by the contemporary advances of "biotechnology"; it is based on almost as empirical and experimental approach as that practiced by the good old alchemist some three or four centuries ago. It is the power of scientifically based predictions that leads us to the best results and the optimum process configurations.

It is the true industrial bio-TECHNOLOGY that this book attempts to address, bringing into it some basic rudimentary methods of process description and optimization based on the magic of the mathematical equation. This does not mean that the bio-scientists among us should stop reading at this point. Simple differential calculus is the backbone of this volume. The concept of mass balancing is summarized in Part II for those who are not used to this most useful and classical basic engineering tool. An extensive and descriptive case study of a selected bio-process in Part III elucidates the concepts of very pragmatic mathematical modeling of the bioreactor systems outlined in Part I.

While the individual concepts dealt with in this volume are of a rather basic nature to the specialists in individual areas involved, it is actually the interdisciplinary nature of the bioprocess field that presents the challenge. Recent advances in these individual areas now make it possible to approach the exciting interdisciplinary task with reasonable confidence. The accumulated knowledge of biochemical microbial pathways, and the experience with description and optimization of chemical reactors, developed in the last three or four decades, is catalyzed by the contemporary power of small, extremely fast and accessible computers loaded with software of powerful mathematical routines. The result is a scientific environment where a qualitative leap can be taken in attempts to quantify some bio-catalytic processes; the industrial ones being of a special interest.

This volume is meant for those who are dealing with the bio-process elements in the laboratory or on a large scale. It is meant for the engineer as well as for the science student, because it is in between the classical fields where the interdisciplinary challenge is, and where the opportunity beckons. Forget the traditional boundaries of scientific disciplines you were inoculated with at school and enjoy the new world of interdisciplinary excitement. It is the energies of this excitement that will bring you through this volume and into the new world of more exact scientific and technological endeavours in biotechnology. It is perhaps time to prepare to leave the age of (bio)-alchemy. The old saying is that every long trek starts with the first step. We hope that this book could be your first step.

The Authors.

CONTENTS

PART I

MATHEMATICAL MODELING OF MICROBIAL PROCESSES

1. INTRODUCTION

For a proper design and operation of fermentation processes, microbial ore leaching, biological wastewater treatment, bioconversion of solar energy by (green) microorganisms, and for other numerous and varied processes of biotechnology, it is essential to know and to be able to quantitatively describe the key process variables relevant to the system kinetics. Such information serves as a basis for deriving an optimal process design and for developing its optimal operation. While in the chemical reactors the process kinetics reflects the reaction rates on a molecular level, microbial process dynamics is a result of relationships between the living microbial cell and its environment affecting the biochemical-physiological activity of the microbial population and thus the results of the whole bio-process. Dynamics and efficiency of the microbial process can be manipulated by the choice of microbial culture and by the physico-chemical environmental factors. An optimal bio-process results from combining the best choices in both areas. Without a suitable microbial strain it is not possible to realize the desired process and, similarly, by using inappropriate process conditions only very low product yields can be obtained even when high-production strains are employed. The methodology of strain selection, its genetic manipulation and optimization of production parameters has been traditionally based on very extensive experimental work, diametrically different from the methods of engineering optimization of a process with regard to its operating parameters.

Considering extremely high costs of industrial-scale experimentation, microbial process engineering approach to experimental work can make an efficient use of laboratory-scale experimentation employing a scaled-down "model system". A geometrically scaled-down copy of the process equipment or a sequence of operations can serve as a model to study the process. This study can be facilitated by developing an analogy of the system, for instance an electrical analogy, or even a mathematical abstraction (math-

ematical model) enabling simulation of the behaviour of an actual process by computations. In most cases, every model represents a certain approximation of the real system and represents a compromise between the high costs and complexity of experimentation with a real large-scale system and the ease of carrying out a smaller-scale experimental study.

This text will mainly deal with problems associated with the application of mathematical modeling methods as a tool of systems analysis in the field of biotechnology. The text is divided into major sections dealing respectively with the methodology of composing mathematical models of bioreactor performance, the types of material balances pertaining to the bioreactor system and, eventually, elaborating on the principles discussed, there is a comprehensive case study where different bioreactor arrangements are modelled and computer simulation of their performance demonstrated. Numerous examples and problems solved throughout the text make the comprehension of the concepts dealt with easier to understand and absorb.

The text has been prepared with broad and interdisciplinary readership in mind. The process engineer will find the concepts more familiar, however, his biochemical and microbiological background has to have been sufficiently developed. The biologist, on the other hand, needs to have basic preparation in integral and differential calculus and the section on mass balances will smooth his entry into the basic area of mathematical modeling of biosystems. In either case, open minded interdisciplinary curiosity, pragmatic approach and unsuppressible desire to be at the "cutting edge" of the contemporary development in new and rapidly expanding areas of biotechnology are the basic requisites for enjoying this text which is to assist in further development and application of the powerful methodology for study and optimization of bioreactor systems in the laboratory as well as in large-scale operation. After all, it is the technology component which is to ultimately fulfill the promises and expectations of the fascinating, new and highly interdisciplinary field of biotechnology.

2. SYSTEMS ANALYSIS APPROACH TO THE MATHEMATICAL MODELING OF FERMENTATION PROCESSES

Systems analysis is a basic method for description of complex phenomena and interactions among observed variables in the process under study[17]. The unified strategy for analysis of an arbitrary process determines the strategy for process optimization. By the process systems analysis we refer to the application of scientific methods to the recognition and definition of process-related problems and the development of procedures for their solution. In practice, for a fermentation process system, this approach is represented by several basic steps:

a) mathematical specification of the problem for the given physicochemical, biochemical and physiological conditions;
b) detailed strategy development resulting in obtaining adequate mathematical model(s) representing the given process;
c) synthesis of results and design of the optimization strategy for process control.

The biological process denotes an actual series of operations and interactions of non-living materials with living matter. Figure 2.1 presents a simplified summary of interactions and links between "microbial process engineering" and other science branches.

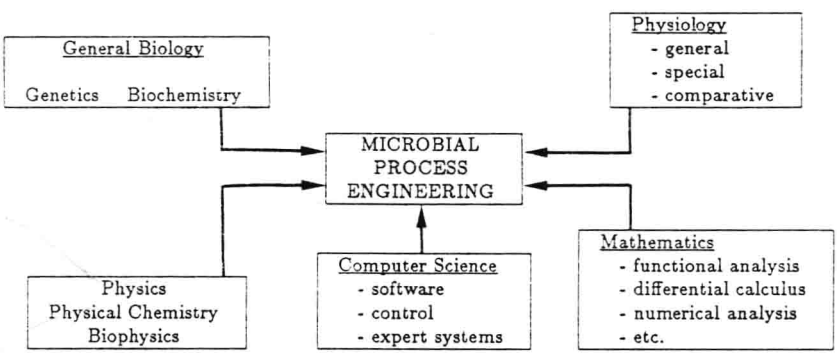

Figure 2.1: The place of "Microbial Process Engineering" among the established science disciplines

Process engineering mostly deals with observed macroscopic kinetics and stoichiometry of biological processes. The kinetics and stoichiometry are based on physiological studies but the theoretical background is developed from enzyme kinetics, metabolic pathways and sometimes on the basis of genetic laws.

The process system state variable is the quantity which can represent an imaginary coordinate in the "state space". Such variables can be determined either by direct measurement (biomass concentration, temperature, pH, etc.) or it can be of an indirect nature. That means that its value is calculated from other measured variables (yield coefficient, RQ, specific growth rate, etc.).

The state of the system is determined by the set of system variables and their corresponding respective rates of changes. Process parameter is a property of the system or its environment that can be assigned arbitrary numerical or linguistic values; also it is a constant or a coefficient in an equation often based on and derived from some assumption such as "ideally mixed", "normal behaviour", "loss of viability", etc.

Simulation is the study of the system or its part(s) by manipulation of its mathematical representation or its usually smaller physical model.

Process analysis involves an examination of the overall process, alternative technological variants and also eventually their economics. There are two main tests in the biotechnological industry with which engineers are ultimately concerned: the optimal operation of an existing plant and the design of new or modified technologies. In the area of operations, both control and optimization of the system performance stand out as two of the main functions of great concern to the process engineer. From a general viewpoint, systems analysis and process simulation have the following benefits:

A) **Extrapolation**. With a suitable mathematical model it is possible to test extreme ranges of operating conditions and also it is possible to establish critical patterns in the performance of the real process.

B) **Study of commutability and evaluation of alternative policies.** New factors (such as use of immobilized cell reactor, or novel bioreactor design) or elements of process equipment can be introduced and old ones removed while the system is examined to see if these changes are compatible. Simulation makes it possible to compare various proposed designs and processes not yet in operation and to test hypotheses about systems or processes before acting (as in the case of the continuous-flow cell retention fermentor with bleeding of the whole broth).

C) **Replication of experiments** by simulation makes it possible to study the effect of changes of system variables and process parameters.

D) **Test of sensitivity and system stability** to disturbances in basic process parameters can be examined.

E) **Optimal control and economic experimentation** can be studied leading to the optimal process design quickly and economically. A study of this sort with a real plant would be extremely risky, expensive and cumbersome involving costly large scale experimentation and design changes.

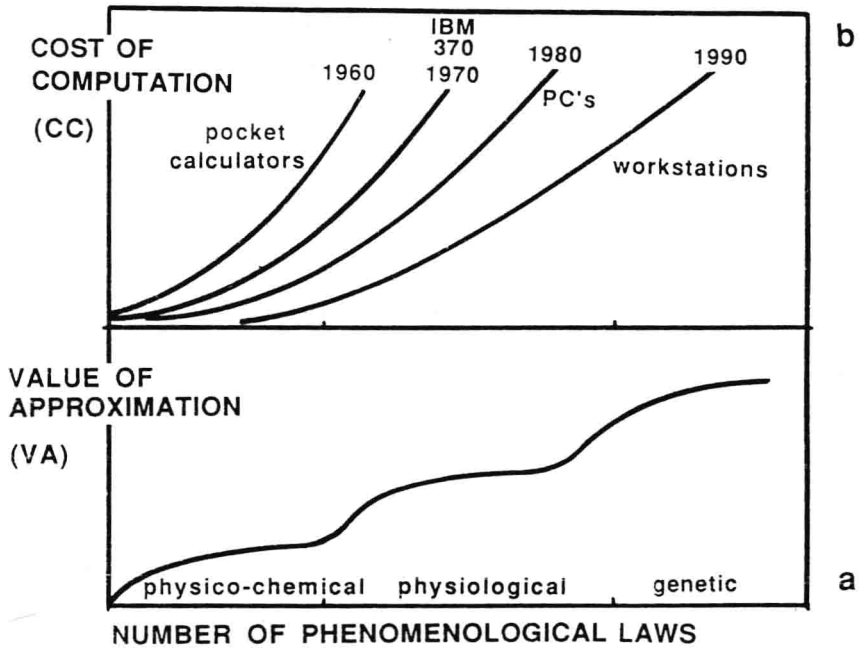

Figure 2.2: The value of approximation by the mathematical model (a) increases with its complexity. The costs of solution (computation) (b) have been gradually decreasing as the computer power is on the rise.

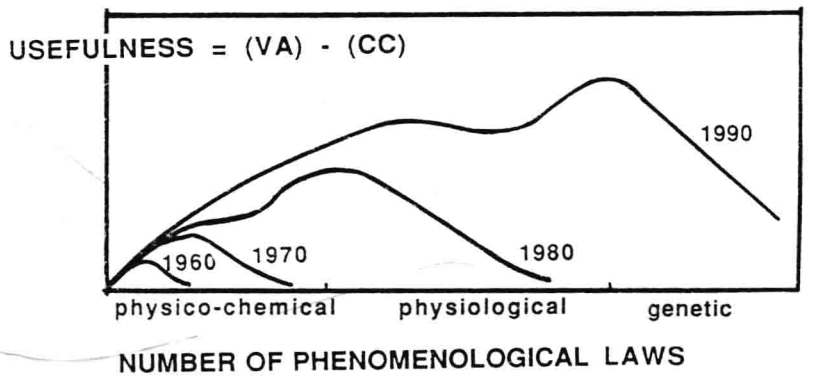

Figure 2.3: Model usefulness as a function of the number of phenomenological laws and time.

Modeling is the process in which the analyst constructs a set of mathematical relationships together with boundary and initial conditions that are isomorphic to relationships among the process variables. Because of the complexity of the real process (physico-chemical, physiological, biochemical and genetic laws) and the mathematical limitations, whatever model is developed is bound to be highly idealized and generally gives a faithful representation of only a few of the properties of the process. The first model is often a simple version of the mass conservation law. On the basis of this model the analyst usually attempts to detect its principal deficiencies. Several models are usually composed before one is established that satisfactorily represents those particular attributes of the process that are of interest. The typical error of all beginners in model building is that they try to include all available information in the model and the product of this effort is a "monster" which is difficult to use in process simulation. The model formulation has to be a compromise between reasonable complexity and desired economy of the solution.

Figure 2.2 compares the approximation ability of the model given by the number of used phenomenological laws and the cost of solution. If no phenomenological law is used in model building the "black box" approach is used for description of the system behaviour. The extrapolation ability of such model is very low. When only the physico-chemical laws were applied, the model could represent an optimal compromise in the era of the mid-seventies. Because of the lowering of cost of model solution by a new generation of software and hardware, the new generation of "physiological oriented" models represent the top of optimal model designs in the eighties. It is clear that further inclusion of genetic, biochemical and biophysical laws (eg. quantum biochemistry and molecular mechanics of protein action) will be in the center of interest of model designers in the upcoming era where the decrease in the cost of solving the model equations will be coupled with the application of multiprocessor "supercomputers" in scientific research. This will allow the design of new efficient algorithms which will make the solution of quantum biochemistry and biophysics problems of microbial physiology possible.

Figure 2.3 outlines the dependence of model usefulness (approximation ability of model vs. cost of model solution) as a function of the number of phenomenological laws and time. In Figure 2.3, the top of the curve in 1970 represents the class of models based on the idea of Monod model for microbial growth and production. The top of the curve in 1980 represents the generation of models with distributed parameters for modeling of tower fermentors, immobilized cells and enzyme reactors, etc. The top in 1990 can be achieved when physiological laws of macroscopic control of physiological functions will be applied. The top of usefulness at the end of the century means the implantation of other biological laws on different levels of the system in simulation models.

The conversion of raw starting materials into valuable products taking place in a biochemical system can be enclosed in a technological system of a bioreactor where most often living microbial cells represent the biological catalytic conversion device[2].

The degree of the overall complexity of a microbially catalyzed process is determined by the complexity of mutual relationships and interactions of the environment and the structured live matter while growing, utilizing and accumulating microbial metabolites. An appropriate mathematical description necessary for composition of a mathematical model has to respect the most important of these relationships and interactions. In case of complex systems, the systems analysis methodology recommends a break-down of these systems into individual sub-systems interconnected by well defined relationships which for microbial systems are usually determined by mass and energy transfer rates between individual sub-systems.

In contrast to technical systems where the subsystems are dimensionally comparable, in microbial processes this arrangement is hierarchical, thus, several subsystems on a certain hierarchical level make up a new subsystem on a higher hierarchical level. Figure 2.4 shows a possible alternative of breaking down a microbial process according to the hierarchical principle given by more or less natural boundaries. In the following paragraphs individual hierarchical levels, which could be distinguished in microbial systems, are briefly discussed.

Hierarchical Levels in Microbial Systems

(I) The first hierarchical level is represented by subsystems concerning molecular or enzyme-catalyzed reactions. This group of subsystems includes all simple catabolic and anabolic reactions, reactions concerning material transport across the cell membrane, also synthesis and decomposition rates of macromolecules involved in catalytic activity, information transfer, energy storage, or those macromolecules having an important structural role. Connections among individual subsystems are determined by the reaction stoichiometry, mathematically usually expressed by the stoichiometric matrix of the reaction scheme. Mathematical models composed on this level, however, are very complex and rarely used. They are mainly encountered in basic research in the fields of biophysics, pharmacokinetics and metabolic disorders. For formulation of mathematical models concerning fermentation processes it is usually assumed that a certain subsystem determined by a reaction sequence between one state and another can be described with adequate accuracy by simplified kinetics based on rigidity or stoichiometric relationships and on the principle of a "bottleneck". These are the reasons why models of fermentation processes are usually derived from subsystems on a second hierarchical level.

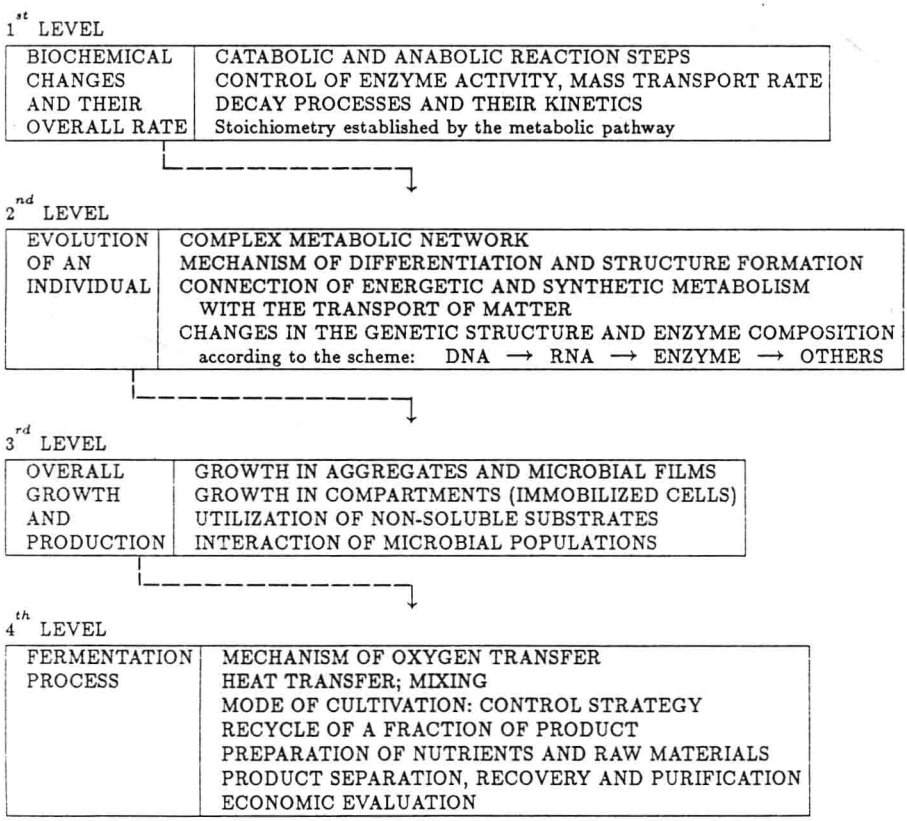

Figure 2.4: A scheme of a fermentation process break-down by a systems analysis method.

(II) The second hierarchical level is characterized by individual parts of metabolism, such as glycolysis, proteosynthesis, substrate transport, etc., being perceived as subsystems making up a complex which reacts to the outside perturbance by changing the rates of growth, substrate utilization and product formation. Models of microbial growth on this level may be represented by either one or several metabolic subsystems. Harder and Roels[25] in their review addressed the question of usefulness of dividing the growth model into a certain number of subsystems. They showed that the number of subsystems in the model depends on the length of relaxation times given by the rates of diffusion ($10^{-5} - 10^{-4}$ s), of enzymatic reactions under allosteric control ($10^{-4} - 10$ s), of RNA synthesis ($10 - 10^{3}$ s), and also by the reaction times concerning the changes in enzymatic concentrations in the cell ($10^{3} - 10^{5}$ s). Considering that rates of diffusion fluxes are from the evolutionary standpoint, according to Snoll[74], counterbalanced by the enzymatic activity under allosteric control, it is possible to include these mechanisms, from the systems analysis standpoint, in one type of subsystems. The overall effect is that for a complete description of the dynamic response of the culture growth to outside perturbances it is quite adequate to use growth models consisting of three dynamically different systems[60].

The three dynamic subsystems differ in relaxation times, i.e., in the dynamics of simple reactions, in the dynamics of RNA synthesis, and in changes of enzymatic concentrations. The dynamic subsystem of RNA synthesis is particularly expressed in transient phases of fermentation, usually in a negative sense as exemplified by the lag phase following inoculation. This effect can be at least partially eliminated by an appropriate preparation of inoculum which is reflected in a model simplified to only one or two subsystems. A non-structural process description by a mathematical model can be used for shorter cultivations when the enzyme concentration can be considered as constant and the growth dynamics is practically dependent on the kinetics of simple reactions connected with catabolism. The non-structural description of the growth dynamics is the most frequent one even though it is not methodologically quite appropriate when used in the transfer of a process from a batch to a continuous-flow culture regime where the long-term changes in culture dynamics may be particularly pronounced in the elemental composition of microbial biomass as well as in enzyme concentrations and metabolic activity of the culture. The use of growth models segregated into several subsystems is, from the systems analysis point of view, an essential means for description of the dynamics of adaptation and selection having an influence on enzymatic composition and metabolic activity in cultivations with long retention times. When modeling cultivations with short retention times the most important aspect is represented by the requirement for simplicity of the mathematical description leading usually to a non-structured mathematical growth model. Apart from the growth dynamics, the model should also include the dynamics of the environment which is an equally important non-living part of the microbial culture.

(III) The third hierarchical level in composing a model of a fermentation process is reflected in modeling of mutual relationships and links between or among microbial strains in mixed population cultivations of the predatory nature when one species serves as a "substrate" for another, or when two species on the same trophic level compete for the same substrate. Modeling of relationships between morphologically different individuals of the same species or those who differ in age also belongs in this category. It is also necessary at this stage to elucidate the effect of growth in colonies or aggregates on the overall growth rate. In practice, these problems are encountered when dealing with phenomena taking place in microbial colonies, microbial films or other natural or artificial aggregates such as cells immobilized in gels or pellets produced by higher microorganisms. These aspects are described in more detail by Ramkrishna[57] or Atkinson[2]. The use of this modeling level is justified only in cases where the above mentioned phenomena affect the overall production rate as the case may be in reactors with immobilized cells, with biological wastewater treatment systems, or with production of certain metabolites in reactors containing purposely cultivated microbial pellets. The segregated models, when compared to non-segregated ones, are considerably more complex and more difficult to solve which makes their use in fermentation technology rather limited to cases when the level of production depends significantly on the age distribution of individuals comprising the culture. These types of models are very significant, however, in the case of systems analysis applied to complex systems because they enable a sensitive simulation of conditions for ecological equilibrium and stability of microbial subsystems in natural environments. These problems are closely related with applications of the fourth level of modeling which then also incorporates system dynamics and properties of the environment.

(IV) The fourth hierarchical level in modeling microbial system is characterized by linking the overall microbial growth and production rates with the dynamic balance of the environment distributed in space with all the attributes of the microbial environment such as kinetics of mixing, heat and mass transfer together with boundary conditions characterizing the intensity of mass and energy exchange with other subsystems, i.e. operations comprising the entire fermentation production process including medium preparation and product recovery.

When formulating a model of a microbial process, feasibility is a guiding principle. A very frequent mistake committed by those with little experience in this field is creation of a very complex model including different approaches available in the literature, disregarding their relevance to the overall goal which should always be the simplest and yet adequately accurate way of describing the real process which would enable its simulation by calculations. Such a model can then be conveniently used for the prediction of optimal operating conditions of a technological process as a whole.