Energetics and kinetics in biotechnology

J.A. Roels

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J.A. Roels

Professor in biotechnology
Delft University of Technology, and

Gist-Brocades NV, Research and Development,
Delft, the Netherlands

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Preface

Optimal utilization of organisms and parts thereof in large-scale processes requires process optimization and this in turn calls for a quantitative and often basic understanding of the energetics and kinetics of biological transformations. In this respect the current developments in bioprocess engineering, being the process engineering part of biotechnology, are analogous to those which have taken place in the more mature chemical engineering. In the chemical industry the large and wide-scale applications of chemical processes induced a great number of studies regarding the development of relatively simple models describing with an acceptable accuracy the often very complex reality of the various processes. This with the objective of enabling reactor design and process optimization.

In the last two decades a growing number of publications appeared on the extension of chemical engineering kinetics and energetics to the description of biotechnological processes and quite a number of useful calculation techniques has been developed. However, the area is rich in pitfalls and the treatments and models are error prone. In the present work a generalized sound theory is developed from basic physical principles and the literature is put in a proper perspective.

Extensive data on the stoichiometry and energetics of microbial growth and product formation are gathered, and correlations and generalizations are developed. The theories underlying the energetics and kinetics are dealt with in great depth. The problems and prospects of the construction of more complicated models are clearly indicated.

The state of the art of the kinetics of biological processes is analyzed and the most useful general models are clearly treated. Great attention is particularly devoted to error prone areas. Of great practical value is the presentation of the general models of biological processes. This fine text and reference book will surely find its way on to book shelves of all professionals dealing with biokinetics and bio(process)technology. This work is a must for both scientists and process engineers in industry, institutes and universities who are faced with this most exciting technology. In addition it will serve as a valuable text book for advanced university courses.

Son, the Netherlands, 1982
Prof. Dr. Ir. H.A.C. Thijssen
Visiting Professor in Food Technology, Eindhoven University of Technology

Contents

CHAPTER 1

Introduction

1.1. Mathematical models

An important aspect of the methodology of present day physics exists in the construction of mathematical models of aspects of the behaviour of real systems. Scientific progress is made possible by comparing the results obtained from mathematical manipulations with the model with the results of experiments on the real system. This generally results in a cyclical process. A schematic representation of the process is given in figure 1.1.

In general one starts with the formulation of a verbal model of the process. Such a verbal statement could, as an example, read: If the substrate concentration in the medium is increased the specific growth rate of the organism present also increases, however, the increase in specific growth rate becomes progressively less if the substrate concentration level is higher. This model can then be translated into a mathematical expression, and one of the possible equations could read*:

$$\mu = \mu_{max} \frac{C_s}{K_s + C_s} \tag{1.1}$$

This equation exhibits indeed all the properties which were indicated in the verbal representation of the model. It is the well-known Monod equation for the substrate concentration dependence of the specific rate of growth. It is extensively discussed in chapter 9. This equation can be used to construct a model of, for example, batch growth of microorganisms and can e.g. be used to calculate the time dependence of the concentration of biomass in the culture. If these predictions are compared with measurements of the biomass concentration in batch growth the validity of the model can be tested. If the concordance between the model predictions and the measured biomass concentration versus time is satisfactory the model is accepted and can be used for a variety of purposes to be discussed later on. If discrepancies between model predictions and measurements exist the model is rejected and, as an example, the verbal model is refined.

It should be emphasized that, in bioengineering as well as in other engineering disciplines, reality is much too complex to be modelled in all its intricacies. The model which would be

* See p. 6 for explanation of symbols.

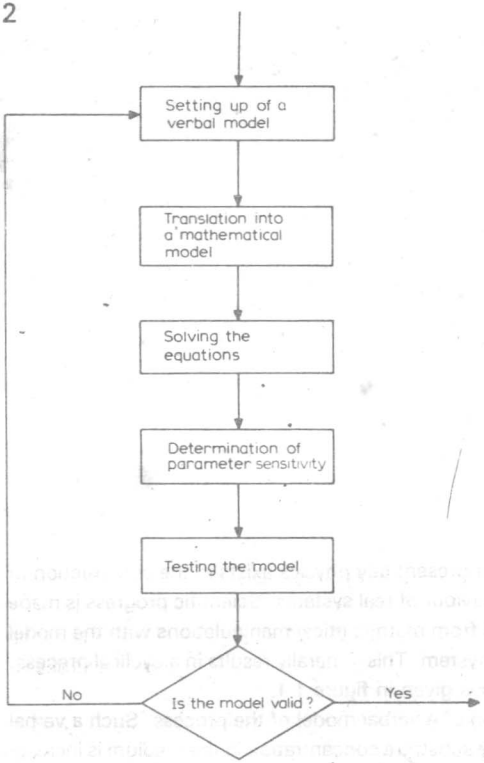

Fig. 1.1. Flow sheet of the construction of a mathematical model. (From ref. 2.)

obtained would be too complex and become scarcely more easy to handle than reality itself. Furthermore, a complex model often contains a large number of parameters, which are generally not known, and must be determined experimentally. Therefore a reduction in the complexity of reality is necessary. This should be guided by universal principles such as a comparison of the relaxation times of system and environment (see chapter 8), and by the application for which the model is designed, as well as the possibility of experimental verification of the model.

1.2. Kinetic models in biotechnology

In chemical engineering the application of mathematical models to the design, operation and optimization of chemical reactors and integral plants is widespread. As early as 1965 Van Krevelen [1] presented an integral picture of the structure of chemical reaction engineering at the Third European Symposium on Chemical Reaction Engineering. The structure he presented can today, in 1981, be applied to biochemical reaction engineering without significant modifications. Figure 1.2 presents an attempt to do this. The macroscopic description of systems, the central part of figure 1.2, as well as of the structure of reaction engineering, is based on the possibility of treating systems in terms of so-called macroscopic variables, e.g. concentrations of chemical substances, amount of energy, temperature and pressure. The

so-called continuum theory, which will be more extensively discussed in section 1.3, translates the behaviour of the objects composing the system, e.g. molecules or microorganisms, into variables in which the corpuscular nature of the system is no longer considered. The basic macroscopic theory is the subject of chapter 2. In chapter 11 the relationship between the continuum approach and the more fundamental corpuscular theory is analyzed.

The basic tool of the macroscopic description is the balance equation. For each so-called extensive quantity, i.e. a quantity which is additive with respect to parts of the system, a balance equation can be formulated, and its structure is always given by [2, 3]:

$$\text{Accumulation} = \text{Transport} + \text{Conversion} \tag{1.2}$$

As can be seen, two types of mechanisms are distinguished by which the total amount of an extensive quantity in the system can vary: transport towards the system and conversion in transformation processes in the system. The balance equations result in the so-called state equations of the model, which describe the time dependence of the state variables of the system. As is clear the construction of the balance equations depends on two types of theories: information on the rates of transport of chemical substances to the locale of the chemical or biochemical transformation processes taking place in the system; and information concerning the so-called micro-kinetics of the transformation processes. Together with the transport phenomena models, the micro-kinetic models constitute a macro-kinetic model of the behaviour of, e.g., a bioreactor or an ecosystem. Such a model can be used in a variety of applications, including reactor design and optimization.

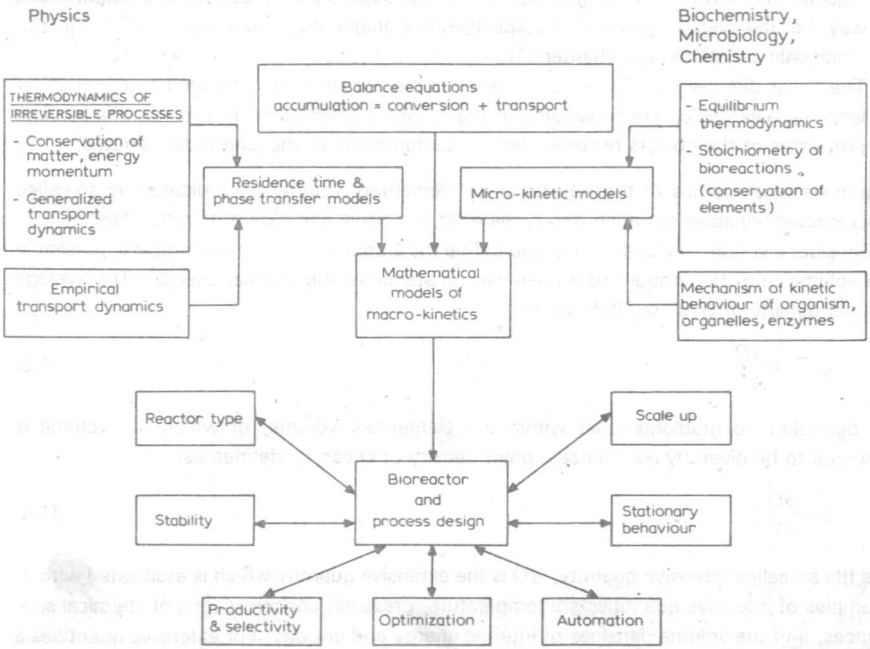

Fig. 1.2. The structure of (bio)chemical reaction engineering. (From Roels, J.A. (1982) J. Appl. Chem. Biotechnol. 32, 59.)

In this book I shall concentrate upon the construction of micro-kinetic models of bioreactions. The integration of transport phenomena models and micro-kinetic models into a macro-kinetic model is beyond the scope of the present work. In addition to the macroscopic theory and its implications for the functioning of organisms and enzymes, information concerning the mechanisms involved in bioreactions, as it is supplied by the growing body of knowledge in microbiology and biochemistry, constitute the ingredients of the models.

1.3. The continuum theory

The molecular nature of matter and the discrete properties of chemical substances and organisms are accepted principles in present day physics and microbiology respectively. It is therefore clear that the fundamentally correct treatment of reality would exist in the construction of so-called corpuscular models (chapter 11). In an important body of approaches to reality, however, the corpuscular nature is ignored. This is especially the case if the object of the study is not associated with the behaviour of individual particles or microorganisms but with the overall behaviour of a large collection of such objects. In this instance a macroscopic treatment is employed and a so-called elementary volume introduced. All matter present in the system is assumed to consist of a large number of elementary volumes, which completely fill the system. The elementary volume has the following properties.

1. The elementary volume is large enough as to contain a sufficiently large number of objects. In this way the average properties of the objects can be defined in a deterministic way, i.e. the average quantities are specified in a unique way and are no longer subject to stochastic variations (see chapter 11).
2. The linear dimensions of the elementary volume are small as compared to the smallest length scales, which are considered in the system's description. In this way the average properties of the objects become continuous functions of the length coordinates.

The average properties of the objects in an elementary volume are indicated by so-called macroscopic variables of which two types exist, intensive and extensive properties.

Consider a volume ΔV of a system and define the amount ΔQ of a given property present in the volume (e.g. the amount of a chemical substance or the internal energy). The average volume density of Q is now defined as:

$$q_{(av)} = \frac{\Delta Q}{\Delta V} \tag{1.3}$$

By definition no gradients exist within the elementary volume, of which the volume is assumed to be given by δV. Hence a point density of Q can be defined as:

$$q = \frac{\delta Q}{\delta V} \tag{1.4}$$

q is the so-called intensive quantity, ΔQ is the extensive quantity which is associated with it. Examples of intensive quantities are temperature, pressure, concentrations of chemical substances, and the volume densities of internal energy and entropy. For extensive quantities a balance equation according to eqn. (1.2) can be constructed.

1.4. Conserved and non-conserved quantities

For the general case the balance equation for an extensive macroscopic quantity contains a transport and a conversion contribution. In a number of systems, however, a number of quantities occur which have the property that they cannot be produced or consumed in the transformation processes open to the system. Hence the conversion term disappears in eqn. (1.2). The balance equation then takes the simple form:

$$\text{Accumulation} = \text{Transport} \tag{1.5}$$

For the systems normally encountered in bioengineering, the amounts of the various chemical elements are conserved quantities. This of course also applies to the systems encountered in chemical reaction engineering. The principle of the conservation of chemical elements is the basis of the stoichiometry considerations of chemical reaction engineering and has become a widely accepted tool in that discipline. However, the application of stoichiometry to reactions in which functioning organisms appear is somewhat more involved, although basically the situation is completely the same as in simple chemical reactions.

At a first view the problems involved in the application of stoichiometry considerations to a growing organism, which is involved in a product formation process, are quite formidable. In the organisms a multitude of chemical reactions occurs between a variety of chemical compounds. Hence an approach in which the stoichiometry of each and every reaction taking place is considered is obviously not feasible. It can be shown, however, that a more simple approach is possible and that a very powerful tool for the analysis of the metabolism of organisms results. After the basic theory has been treated in chapter 2, the development of applications is undertaken in chapters 3 and 4.

1.5. The first and second laws of thermodynamics

Thermodynamics is, in its classical form, a typical example of a macroscopic theory. The theory of equilibrium thermodynamics is an accepted tool in chemical reaction engineering and of course the constraints posed by the first and second laws of thermodynamics also apply to processes in which organisms, or parts of organisms, like enzymes, appear. In its classical form equilibrium thermodynamics cannot be applied to so-called open systems, i.e. systems, which exchange mass as well as energy with the environment. Unfortunately, however, microorganisms are open systems and the same applies to chemical reactors, which are used in continuous operation. The theory of choice to treat such systems is non-equilibrium thermodynamics, a theory of which the development has progressed considerably during the past four decades.

The most important principles, on which non-equilibrium thermodynamics are based, exist in the formulation of constraints to the conversion terms appearing in the balance equations for two extensive quantities, energy and entropy. The first law of thermodynamics states that energy is a conserved quantity, i.e. the conversion term appearing in the balance equation for energy is zero. The first law generalizes this constraint to apply irrespective of the nature of the system in which the process takes place. The second law of thermodynamics states that the entropy production in any possible process must exceed zero. The combined application of the first and second laws of thermodynamics allows the definition of a fundamental

measure for the efficiency of the energy transformations inside a system. This is the so-called thermodynamic efficiency.

In chapter 2 the basic formalism of non-equilibrium thermodynamics is treated, in chapters 3 and 4 it is applied to the energy transformations in organisms, in chapter 5 it is applied to the analysis of the efficiency of oxidative phosphorylation, the process in which metabolic energy in the form of ATP (adenosine triphosphate) is generated from metabolic reducing power. In chapter 6 the theory is applied to the analysis of patterns of chemical reactions.

1.6. The kinetic description of transformation processes

Stoichiometric considerations and thermodynamic considerations allow an analysis of processes without the introduction of detailed kinetic assumptions only up to a certain level. If one wants to proceed further, kinetic models of the transformation processes in the system are needed at a certain stage. The problem, which is encountered in the construction of constitutive or kinetic equations of transformation processes is that a macroscopic kinetic theory of the generality of thermodynamics does, at present, not exist. Kinetic descriptions still have, certainly in the realm of bioengineering, a number of the aspects of an art. A problem encountered in the kinetic description of processes is that generally a large number of mechanisms can be distinguished which underlie the transformation processes inside a system. A reduction of the complexity of reality is therefore necessary in many instances. A strategy to obtain a simplified model is developed in chapter 8. In chapter 7 constitutive equations for single enzymes and sequences of enzymic reactions are treated. In chapters 9 and 10 approaches towards the kinetic description of functioning organisms are developed. In chapter 9 so-called unstructured models are considered. These models are based on a very rigorous simplification of the complexity of a living organism. It is assumed that the amount of biomass present in the culture provides sufficient information about the activities of biomass. Changes in the internal structure of the organisms are considered not relevant. In chapter 10 the extension of the theory to models, in which some aspects of the internal structure are considered, is performed and structured models are treated. Finally, in chapter 11, the corpuscular description is briefly treated and its consequences for the correct formulation of macroscopic kinetic models are discussed.

List of symbols

C_s	Substrate concentration	mole/m^3
K_s	Monod saturation constant	mole/m^3
ΔQ, δQ	amount of an extensive property	kg, mole
q	volume density of an extensive quantity	kg(mole)/m^3
ΔV, δV	volume element	m^3
μ	specific growth rate	h^{-1}
μ_{max}	maximum value of the specific growth rate	h^{-1}

References

1 D.W. van Krevelen, Proceedings of the Third European Symposium on Chemical Reaction Engeneering. Pergamon Press, Oxford (1965).

2 J.A. Roels and N.W.F. Kossen, 'On the Modelling of Microbial Metabolism'. In: M.J. Bull (Ed.) Progress in Industrial Microbiology, Vol. *14*, p. 95, Elsevier, Amsterdam (1978).

3 J.A. Roels, Biotechnol. Bioeng. *22*, 2457 (1980).

CHAPTER 2

Macroscopic theory for open systems*

2.1. The mathematics of the macroscopic description

In figure 2.1 a system of volume V bounded by the surface S is shown. An extensive property E, of the system as a whole is considered, it is given by**

$$E = \int_V e dV \tag{2.1}$$

In eqn. (2.1) the intensive quantity e is the volume density of the property E. E stands for the total amount of E present in the system. In general two mechanisms may be distinguished, by which the total amount of extensive property E present in the system may vary. E may be exchanged with the environment by transport over the system's boundary, furthermore E may be produced or consumed in processes taking place inside the system. This leads to the verbal formulation of a so-called balance equation for the amount of E present in the system, it is given by

$$\text{Accumulation} = \text{Conversion} + \text{Transport} \tag{2.2}$$

The verbal statement given by eqn. (2.2) can be translated into the following mathematical expression:

$$\dot{E} = \Pi_E + \Phi_E \tag{2.3}$$

In eqn. (2.3) Π_E represents the total net rate of production of E in the processes taking place in the system, Φ_E represents the total rate of exchange of E with the environment. By analogy to the definition given in eqn. (2.1), it is now possible to define the volume density of the rate of production of E as follows:

$$\Pi_E = \int_V r_E dV \tag{2.4}$$

In eqn. (2.4) r_E is the rate of production of E per unit volume. In the same way a surface density of transport can be defined:

* See refs. 1–3.

** See p. 21 for explanation of symbols.

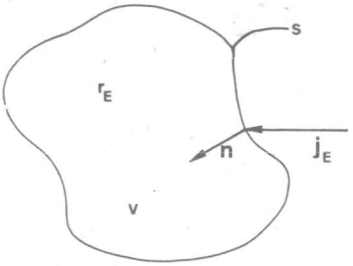

Fig. 2.1. System for the purpose of a macroscopic analysis.

$$\Phi_E = \int_S \mathbf{n}\mathbf{j}_E dS \tag{2.5}$$

in which **n** is the inward normal on the surface of the system and $\mathbf{j}_E$ is the surface density of rate of transport to the system [4].

Combination of eqns. (2.1) and (2.3)–(2.5) results in the following global balance equation for E:

$$\frac{d}{dt}\int_V e dV = \int_V r_E dV + \int_S \mathbf{n}\mathbf{j}_E dS \tag{2.6}$$

Equation (2.6) applies to the system as a whole (macrobalance) as well as to parts of the system (microbalance).

In an important part of the present work only homogeneous systems are considered, i.e. e, r_E and $\mathbf{j}_E$ are assumed independent of the spatial coordinates. Therefore, eqn. (2.6) can also be written as:

$$\dot{(eV)} = r_E V + \phi_E V \tag{2.7}$$

In eqn. (2.7) ϕ_E stands for the total rate of transport of E to the system per unit volume of the system. It is defined as:

$$\phi_E = \frac{1}{V}\Phi_E \tag{2.8}$$

Furthermore, in some instances I shall restrict myself to systems of constant volume, and in this case an even simpler equation applies:

$$\dot{e} = r_E + \phi_E \tag{2.9}$$

2.2. Conserved and non-conserved quantities

It is important to make the distinction between quantities which are conserved in the reaction pattern in the system, and quantities which are not conserved. For a conserved quantity no net production occurs in the transformation processes taking place in the system, or mathematically

$$r_E = 0 \tag{2.10}$$

Hence, for these quantities, the balance equation given by eqn. (2.7) takes the form:

$$(\dot{eV}) = \phi_E V \tag{2.11}$$

Or, if the volume of the system is constant:

$$\dot{e} = \phi_E \tag{2.12}$$

These equations are of great importance to the description of the transformations in systems.

Note 2.1

An important group of examples of conserved quantities with respect to chemical transformations are the amounts of the chemical elements. The conservation principle for the amounts of these quantities provides the basis for the stoichiometry of chemical reactions, a fundamental tool in the description of chemical transformations.

2.3. The balance equation for the chemical state vector of a system [5–7]

A system is considered in which a number of chemical compounds exists. The intensive quantities associated with the total amounts of the chemical substances present are their concentrations. These will be expressed in moles per m^3. If n chemical compounds are present, their concentrations may be expressed as a row vector of dimensionality n:

$$\mathbf{C} = [C_1 \ldots C_i \ldots C_n] \tag{2.13}$$

Each element C_i of the vector **C** represents the concentration of one compound. In the system a number of chemical transformation processes take place. It is assumed that m independent chemical reactions take place. Their rates are given by the m dimensional row vector **r**:

$$\mathbf{r} = [r_1 \ldots r_i \ldots r_m] \tag{2.14}$$

The rates r_i are expressed in moles/m^3s. Each reaction taking place in the system can be represented by a stoichiometric equation. The number of moles of compound j produced per unit rate of reaction in reaction i, is assumed equal to α_{ij}. In this case the total reaction pattern in the system is characterized by the stoichiometry matrix α, an $m \times n$ matrix

$$\alpha = \underset{n \text{ components}}{[\alpha_{ij}]} \quad m \text{ reactions} \tag{2.15}$$

Example 2.1

In order to clarify the nature of the formalism introduced above, the following reaction pattern can be considered:

Reaction 1: $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$
Reaction 2: $CH_4 + \frac{1}{2}O_2 \rightarrow CH_3OH$
Reaction 3: $CH_4 + \frac{3}{2}O_2 \rightarrow CO + 2H_2O$

As six compounds participate, the chemical state vector reads:

$$\mathbf{C} = [C_{CH_4}\ C_{O_2}\ C_{CO_2}\ C_{CO}\ C_{H_2O}\ C_{CH_3OH}]$$