2 Analysis & Modeling

Abstract In this chapter, simple transitions of the components of biological systems are considered, the transitions being essential in any process. The transitions are affected by external stimuli, and that is shown in this chapter as well. Step-by-step modeling helps better understanding. Modeling is also of practical interest. The notations (different from the conventional) used here (abandonment of square brackets, use of a single character without a subscript, expression of the backward transition rate via that of the forward one, use of the probabilities of the states) and the use of computers and the corresponding software allow to obtain the solutions of equations immediately, the solutions being more comprehensible due to the condensation. Also of interest is the viewpoint that a complicated process comprised of the processes proceeding on different temporal scales can be split up, the constituents being considered independent; that ligand binding does not depend on its futurities; that the second ligand is not necessarily an inhibitor. Such an approach is useful in the analysis. Only basic mathematical knowledge is required to understand and model the processes. Mathematics is here just a tool. The computer programs also should be considered similarly.

Keywords Bioprocess analysis • Mathematical models • Model fitting • Model verification • Feedback

2.1 Introduction

The complexity of biological processes is inevitable because of the enormous complexity of the environment and of the systems themselves. Even in the case of a single macromolecule its properties change in response to any change in the environment. That implies the need to analyze the process of change.

Analysis involves “breaking down” the issue. Presumably, a process is adequately understood if its progress can be presented as a sequence of elementary events, the rate of each of them being known.

It seems reasonable to assume the properties of any objects to remain constant as long as the surrounding conditions do not change. Biological processes are assumed to obey physical laws. Clearly, any process is unambiguously characterized by its rate.

The rates are best (formally) described by differential equations. The equations relate (beyond other things) the rates of change of the corresponding quantities with the quantities themselves. A process is unambiguously determined whenever

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A. Juška, *Analysis of biological processes*, DOI 10.1007/978-94-017-7373-7_2

its differential equation is known and the initial conditions are specified. The equation can be solved using corresponding software. Analytical solution (facilitating understanding) is preferable (although not always possible).

A process might be as well described verbally, resulting in its verbal model. In the case of considerable complexity, the description becomes too cumbersome and errors are unavoidable. Schematic presentation of the model makes it easier to be grasped, understood, allows to notice possible discrepancies. On the other hand, such a scheme can be "translated" effortlessly into differential equations.

To define the relationships quantitatively, mathematics uses quantities or values rather than objects themselves. To apply mathematics to biology, the biological objects have to be characterized by their states to which certain quantities (probabilities, concentrations or the like) can be ascribed.

A closer analysis of the process (and the data concerning it) is helpful in suggesting the possible models, requires refining the initial assumptions and notions.

The biological processes of interest are not expected to be simple. Still, keeping in mind the limited accuracy of experimental data, the data can be adequately approximated with rather simple models. (As a matter of fact, the application of more sophisticated models is not justified when more than one model can be in agreement with the data.) That is related to reducing a complex process to a simple one. Often that is acceptable.

A thorough analysis of a process suggests useful simplifications. A complex process sometimes may be split up into separate trains progressing on different time scales, those trains being considered independent. The adaptation of microorganisms to new conditions may be considered to be independent of their division (Chap. 7); the association of the substrate with the enzyme and its enzymatic conversion into a product (Chap. 12) should be considered independent as well.

Analysis might suggest new experiments as well. The technical aspects of the analysis are considered in the next (3) chapter.

2.2 Analysis

Analyzing facts concerning a phenomenon leads to its verbal model, the model remaining still *qualitative*. To facilitate the translation of the qualitative model into the *quantitative* (or mathematical) one, it is convenient to present the former schematically. The scheme (or cartoon) has to be as concise as possible: to contain only elements and symbols of interest (avoiding everything what could distract, even if important from other points of view); multi-character symbols, subscripts or superscripts, brackets and signs of arithmetic operations (in a sense different from arithmetical) should not be used. A simple process of the transition of an object residing in state **X** into state **Y** proceeding with the *relative rate* μ is presented schematically in Fig. 2.1 (top left) where *x* and *y* mean the *quantities* of

the objects (or variables considered by the model). The scheme is a *qualitative* expression of the relationship between the *quantities* and the *rates* of their change. The translation of this scheme into a mathematical equation follows naturally as will be seen in the next section.

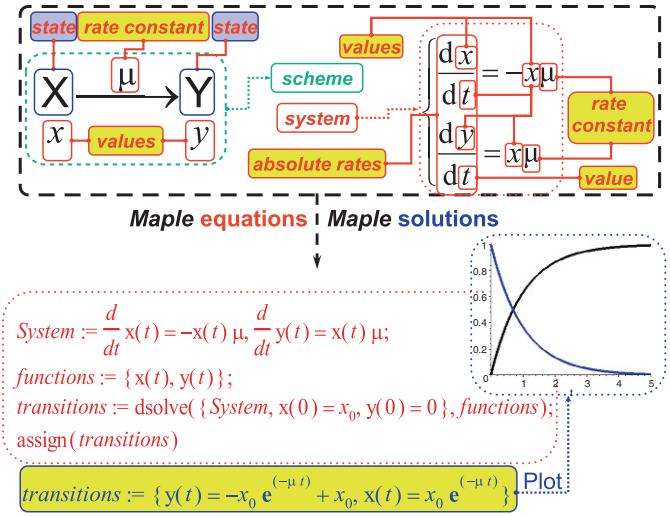


Fig. 2.1 From scheme of the process to differential equations, the solution and plotting by *Maple*

Onward, as stated in the Introduction (Chap. 1), Arial Unicode MS fonts will be used to symbolize the entities, the variables will be symbolized by Latin (non-bold, non-capital, *Italic*), the relative rates, by Greek (non-capital, non-*Italic*) letters. *Curtz MT* (*Italic*) fonts will be used for dimensionless multipliers or ratios. ***Old English Text*** fonts will be used to suggest the propinquity of the objects denoted correspondingly, to avoid identical notations for them and to remind, however, of the differences. Occasionally the *Colonna MT* font will be used as well in special cases.

2.3 Modeling

2.3.1 Unidirectional Transitions

The simplest transition of a physical body from one state to another seems to be its binding (or unbinding) to (or from) another body, the binding (or unbinding) being also the simplest interaction.

From the scheme presented in Fig. 2.1 follows the system of differential equations ($dx/dt = -\mu$ (the *absolute* rate of change of the x quantity is equal to x (the changing quantity) multiplied by μ (above the arrow in the scheme, the *relative* rate or *rate constant* of the change) being with "–" sign when the arrow is directed *from* the changing state but positive when the arrow is directed *to* the changing state, therefore, $dy/dt = x\mu$); the equations can be solved using computers and a corresponding software, the solutions being returned immediately.

The same equations follow also from a simple reasoning. Since the *relative rate* of the *change* in the *quantity* of an object is the *ratio* of the *absolute rate* of the *change* and the *quantity* of the changing object, otherwise, the *absolute rate* is *the relative rate* multiplied by the *changing quantity* (mathematical *value*), or the *derivative* of the *quantity* = the *relative rate* × the *quantity*. Or, symbolically,

$$\frac{\mathrm{d}x}{\mathrm{d}t} = -\mu x, \tag{2.1}$$

where μ is the *relative* rate or the *rate constant*, $\mu > 0$, the "–" sign taking into account the *decline* of the *quantity* or mathematical *value*, x, the *absolute* rate of change here being negative.

All the conditions have to be specified. The model is fully defined under specific (initial) conditions.

The equation can be easily solved manually; it can be expressed as

$$\frac{\mathrm{d}x}{x} = -\mu \mathrm{d}t \tag{2.2}$$

or, integrating both sides of the above equation,

$$\int \frac{1}{x}\mathrm{d}x = -\int \mu \, \mathrm{d}t \tag{2.3}$$

or

$$\int \frac{\mathrm{d}(\ln(x))}{\mathrm{d}x}\mathrm{d}x = -\int \mu \, \mathrm{d}t, \tag{2.4}$$

or

$$\ln(x) = -\mu t + \mathrm{const.} \tag{2.5}$$

Raising e to the power of each side of the above equation results in

$$x = x_0 \exp(-\mu t), \tag{2.6}$$

where $x_0 = x(0)$ corresponds to the initial conditions (it is often convenient to assume $x_0 = 1$ ignoring its dimension).

Since during the transition the total quantity of the both states ($x + y$) remains unchanged, $y = x_0 - x$. Then

$$y = x_0\left(1 - \exp(-\mu t)\right). \tag{2.7}$$

As mentioned above, making use of computers and software greatly facilitates the solution of the equations and model presentation (see Fig. 2.1; note that the graph (as well as the solution of the system of equations) is generated by the software and follows from the scheme on the upper left corner based on the verbal model of the process). Models (2.6) and (2.7) are presented in Fig. 2.2 and, being of fundamental importance, are applicable to any single-step transition proceeding with the relative rate μ. The transitions are analyzed in more detail in Chap. 4 and others.

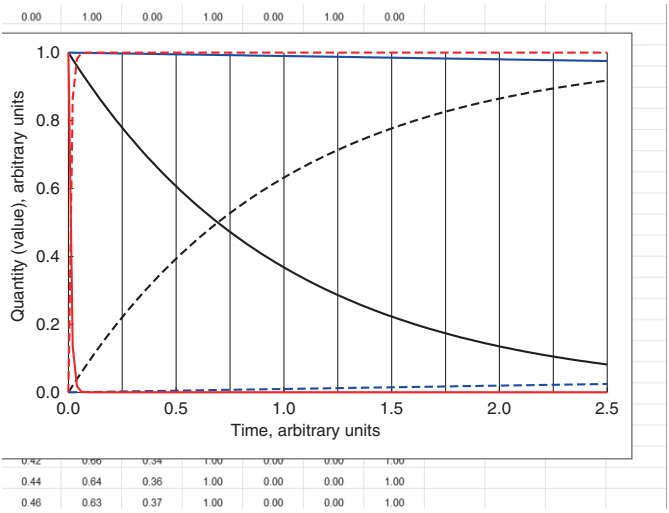


Fig. 2.2 Fraction of *Microsoft Excel* worksheet containing a table and model graphs. Models (2.6) (declining curves) and (2.4) (rising curves) correspond to different rate (or time) constants

2.3.2 Feedback

A transition **X** → **Y** is considered, and the feedback is taken into account. Let **X** and **Y** to be states of biological objects taking part in the transition, x and y,

corresponding quantities or values (which will be considered as variables, both of them depending on time, t), μ, the relative rate of the transition unaffected by the feedback. In the case of the negative feedback, the transition depicted schematically in Fig. 2.3 can be presented also as follows:

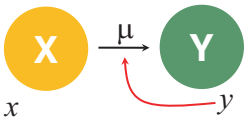


Fig. 2.3 Cartoon depicting transition with a feedback affecting the (absolute) rate of the transition

$$\underset{x}{\mathbf{X}} \xrightarrow{x\mu(1-f(y))} \underset{y}{\mathbf{Y}}, \tag{2.8}$$

where $f(y) \geq 0$; if $f(y) = fy$, the transition proceeds as

$$\underset{x}{\mathbf{X}} \xrightarrow{x\mu(1-fy)} \underset{y}{\mathbf{Y}}, \tag{2.9}$$

i.e. the transition rate will be affected by the result of the same transition. Here, fy is dimensionless, the dimension of f being, therefore, the reciprocal of that of x or y. (The negative feedback, therefore, leads to $y \to x$ or the value of y being kept constant).

2.3.3 Non-Temporary Action of External Stimuli on the Transitions

The effects of external stimuli are analyzed in more detail in Chaps. 8–11. It is clear that modeling the action of the stimulus involves one more dimension, one more independent variable. The dependence on that variable (the former, x variable, not being considered here as external) will be *stationary* rather than *temporal*. To indicate the difference, dissimilar notations will be used, the scheme of interactions (Fig. 2.4), however, remaining rather similar to the original one.

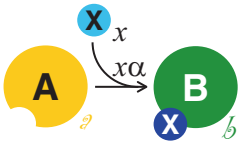


Fig. 2.4 Cartoon of transitions of enzyme or receptor (macro)molecule in non-liganded state, **A**, caused by its interactions with ligand, **X**, leading to state **B** of the (macro)molecule; a and b symbolize relative quantities (concentrations) of the (macro)molecules residing in the corresponding states, x, ligand concentration

Here, **A** and **B** are states of the (macro)molecule depending on x, the external stimulus intensity and transition rate, α (being a constant); neiter of the values not being defined more precisely. The scheme reflects unidirectional, *irreversible* transition. The "final" result of the reaction (at $t \to \infty$) would not depend on x: all the (macro)molecules finally would be in state **B**. A different equilibrium between **A** and **B** states is possible only if the transition is *reversible*. The *backward* transition, therefore, has to be taken into account as shown in Fig. 2.5. Here, β is a rate constant which further is expressed via α, $\beta = \boldsymbol{x}\alpha$, $\boldsymbol{x}$ being a constant of the same dimension as x (not defined more precisely).

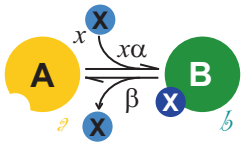


Fig. 2.5 Cartoon of *reversible* transitions of enzyme or receptor (macro)molecule in non-liganded state, **A**, caused by its interactions with ligand, **X**, leading to state **B** of the (macro)molecule; a and b symbolize relative quantities (concentrations) of the (macro)molecules residing in corresponding states, x, ligand concentration, β, the rate constant of the backward transition

It is clear that the necessary step of the enzymatic reaction is the formation of the enzyme–substrate complex resulting in the complexed enzyme, the free one and the product. The complex is considered here as a new state of the enzyme different from the initial one. The transition of an entity residing in one state to another one considered above is applicable here, but now the transition depends on substrate or, rather, more generally, on ligand concentration, another interacting entity not necessarily being an enzyme, it may be any (macro)molecule (see Fig. 1.1). Now the transition depicted in Fig. 2.5 has to be considered. The dependence of the probability of the state of the (macro)molecule induced by the ligand on its concetration will be *stationary* rather than *temporal*. The corresponding equations are based on the above scheme (Fig. 2.5).

It is clear that at equilibrium at any ligand concentration the algebraic sum of the rates of forming a complex of these molecules with the (macro)molecules and the decay of the complex is nil (that makes an equation), and the sum of the states remains constant (it is convenient to suppose it to be equal to unity) making another equation. The system of algebraic equations, therefore, is the following:

$$\begin{cases} -ax\alpha + b\boldsymbol{x}\alpha = 0, \\ a + b = 1, \end{cases} \tag{2.10}$$

whose solutions are

$$a = \frac{\boldsymbol{x}}{x + \boldsymbol{x}}, \tag{2.11a}$$

$$\mathcal{Y} = \frac{x}{x + \boldsymbol{x}}. \tag{2.11b}$$

As above, making use of computers and software greatly facilitates the modeling.

2.4 Preparation of the Data Set to Compare It with the Model

It is assumed that experimental data are presented in a table of the *Microsoft Excel* worksheet or any other spreadsheet ($n \times p_{max}$ (rows and columns)) where the values corresponding to the *independent* variable (depending only on the choice of the experimenter, such as time, concentration, etc.) are arranged in ascending (usually but not necessarily) order (convenient for the further graphical presentation) in a column, the corresponding data of the *dependent* variable (i.e. the results) being presented in other columns, separate columns being allocated for separate replicates. The address of any value (its place in the table) is unambiguously determined, therefore, by corresponding row ($1 \le i \le n$) and column number ($1 \le j \le p_i$, p_i being the number of series) as shown in Fig. 2.6.

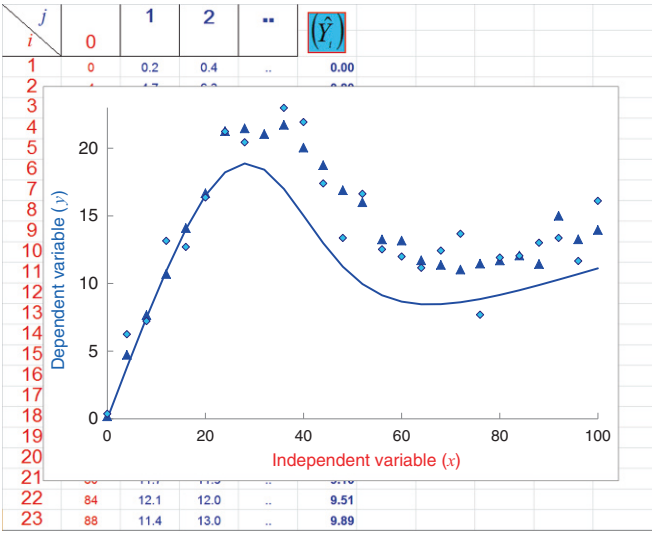


Fig. 2.6 Fraction of *Microsoft Excel* worksheet containing a plot of notional "data" marked differently depending on their series; the curve (a putative model) is plotted as well. The tabulated "data" values are presented in the *C* and *D* columns of the worksheet and those of the model, in the *F* column (under $(\hat{Y}_i)$)

The data points of separate series are marked differently on the plot. The model is also plotted. Its scale (both horizontal and vertical) has to be chosen

appropriately to accommodate the model curve on the graph. The curve has to be comparable to the progress suggested by the sequence of experimental data points. Its fitting to the data points is considered in Chap. 3.

2.5 Discussion

With the progress of analysis, the latter becomes more and more formal, and the process itself becomes of less interest. The problem (technically) becomes other than just biological or (bio)chemical. The main principles, however, remain and have to be kept in mind, and the corresponding instruments (the software in this case) used knowingly.

It has to be noted that the outlook of the curves on graphs depends (among other factors) on the relationships of the parameters and the scales of the graphs. In Fig. 2.2 there are depicted the models (Eqs. (2.6) and (2.7)) with different rate or time constants. The processes proceed essentially the same way, still differing by their rates or corresponding time constants (τ, the reciprocals of the rate constants). The differences as mentioned in Chap. 1 and will be shown in Chap. 12 can be used for simplification. In Fig. 2.7, there are depicted models (Eqs. (2.11a) and 2.11b) in the so-called semi-logarithmic scale with different $\boldsymbol{x}$. It can be seen that the models are fully determined by a single parameter, $\boldsymbol{x}$, their corresponding shape being identical.

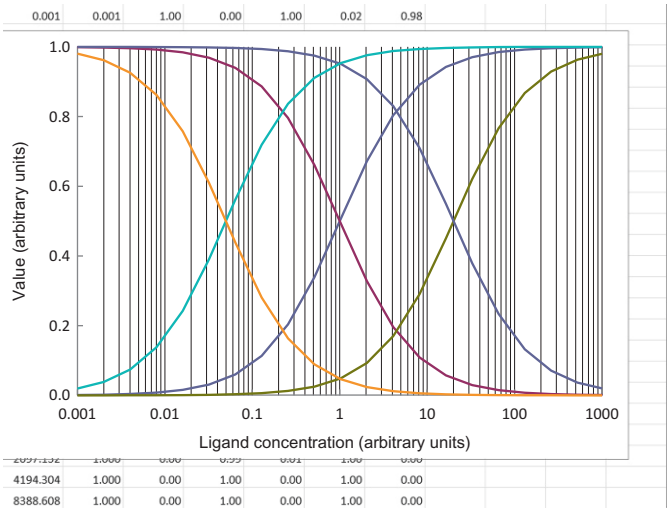


Fig. 2.7 Fraction of *Microsoft Excel* worksheet containing a table and model graphs. Models (2.11a) (declining curves) and (2.11b) (rising curves) correspond to different (macro)molecule–ligand equilibrium dissociation constants ($\boldsymbol{x}$) determining the shift of the curves

As mentioned above, the process of the simple transition is determined entirely by a single parameter, μ. Considering *time* constants (along with the *rate* constants) is helpful in parameter estimation or graphical presentation. Parameter τ has to be in the range of the time scale of the graph or slightly lower than the duration of the experiment (or, otherwise, if there is information concerning the process, experiment duration has to be chosen accordingly), otherwise it may appear visually that no change is modeled. Indeed, if τ is much greater than the greatest value of the temporal axis, the change is very slow (no change visually), otherwise the change is very fast (sudden, following visually no change again). That may be shocking for those with little experience in modeling. A similar awkward situation may arise with plots in the semi-logarithmic scale. Besides, there is no reason to have such an extension of the abscissa axis that on the graph no change can be seen.

There is a good reason to depict the model curves as lines (of any style, colour or weight) *without* any marker (as made possible by *Microsoft Excel*) reserving the markers for experimental data. The marker corresponds to the values of both independent and dependent variables (usually time, concentration or the like and the results of the measurements) ensuring an unambiguous presentation of the data on the graphs. Using markers for model curves does not make any sense, besides, that would make possible, and almost inevitable the visual interference of the model with the data, inconvenient for the analysis. There is no sense, either, to connect the experimental data points, moreover, by *smoothed* lines. Likewise, three-dimensional presentation, if not providing any additional information to the graph, is not justified.

Analysis of a phenomenon involves considering separate facts in addition to the data of special experiments leading to the verbal description of the phenomenon.

As mentioned above, Eqs. (2.2) and (2.6) or (2.7) are fundamental. They model the elementary process of *any* change. Eq. (2.2) contains essential information concerning the process, except for the initial conditions (necessary for the immediate model, i.e. its solution).

The notations used in the scheme (abandonment of square brackets, the use of a single character without a subscript, expression of the backward transition rate via that of the forward one, the use of the probabilities of the states) and the use of computers and the corresponding software, as mentioned above, allow to get the solutions of System (2.10) immediately.

It has to be noted that no special assumptions were made concerning the nature or properties of the independent variable, or stimulus, x, in the scheme of interactions (Fig. 2.5) or Eq. (2.10); consequently, Eqs. (2.11) are rather general: they follow immediately from the scheme bypassing several steps and intermediate equations as in most textbooks and making use of accessible software. For $\boldsymbol{x} = x$, it follows from Eqs. (2.11) that $a = x/(x + \boldsymbol{x}) = 1/2$ and $b = x/(x + \boldsymbol{x}) = 1/2$. From here follows the meaning of $\boldsymbol{x}$: it is equal to the stimulus intensity at which the probabilities of both states of the object under transition are equal, the dimension of $\boldsymbol{x}$ being the same as that of the stimulus x. If the scheme is viewed as enzyme

transition resulting from its interaction with the substrate, **A** being the unbound, **B**, the substrate-bound state and x, substrate concentration, then $\boldsymbol{\varkappa}$ is the (equilibrium) *dissociation* constant, its reciprocal, $1/\boldsymbol{\varkappa}$ being the *association* constant. Equations (2.11) express the main relationships of (bio)chemical kinetis; the relationships are usually referred to as Henri–Michaelis–Menten (Cornish-Bowden 2004; Copeland 2000) or even just as Michaelis–Menten kinetics (Nelson and Cox 2013; Frère 2003) and Kühl (2003) remind that the relationship was established by Henri and published 10 years earlier (in 1903) than by Michaelis and Menten (in 1913).

It should be emphasized that the state **B** does not necessarily mean that of the enzyme-bound ligand (enzyme-bound substrate) which undergoes the enzymatic transition: the ligand may undergo no change except for its binding to the (macro)molecule. The dependence of binding on ligand concentration is modeled by Eqs. 2.11 which hold true for any interacting molecules. It is assumed tacitly (in System 2.10 and usually in textbooks) that the concentration of the ligand x is high enough (usually much higher than that of the other interacting agent (enzyme in the textbooks)), $x >> z$ (the latter being the concentration of the other agent); that will be analyzed in more detail in Chap. 11. It should be noted that the dependences of states **A** and **B** on the concentrations of the ligands (boths x and z), are asymmetric with respect to the ligands, the asymmetry following from the assumed inequality above ($x >> z$). If the (macro)molecule is an enzyme, its efficacy seems reasonable to assume to be non-zero if the probability of its being in state **B** is non-zero as well or $b > 0$. If its role is quite different (e.g., in the case of receptor), both the **A** and **B** states (a and b probabilities) have to be taken into account when determining the total efficacy (being equal to $a\mathfrak{a} + b\mathfrak{b}$):

$$e = a\frac{\boldsymbol{\varkappa}}{x+\boldsymbol{\varkappa}} + b\frac{x}{x+\boldsymbol{\varkappa}}, \tag{2.12}$$

e being the total efficacy, a and b the efficacies of the (macro)molecule in **A** and **B** states. If the (macro)molecule is not an enzyme, $a \geq 0$.

It can be seen from the above equation that considering simple molecular interactions results in a model more general than those considered in enzyme kinetics but reduced to it if the unbound (macro)molecule is inactive ($a = 0$), being active only in bound state. The modification (extension) of the scheme of interactions (Fig. 2.5) results in more sophisticated and useful models (see Chaps. 9–11).

It is recommended to exercise and practise what is presented here, the modeling and comparing (visually) the model with experimental data.

2.6 Conclusions

- The analysis of a phenomenon leads to its verbal model.
- A schematic presentation of the verbal models facilitates obtaining the mathematical ones and does not require any additional assumptions.
- Mathematical modeling is useful in providing means for a better understanding of the phenomenon.
- Modeling is helpful thanks to the very approach.

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http://www.springer.com/978-94-017-7372-0

Analysis of biological processes
Verbal description, schemes, and mathematical models
Juška, A.
2015, XIV, 166 p. 45 illus. in color., Hardcover
ISBN: 978-94-017-7372-0