

CAMBRIDGE TEXTS IN  
BIOMEDICAL  
ENGINEERING

# Biosimulation

Simulation of Living Systems

$$\frac{\partial c}{\partial t} = v \cdot \nabla c + \nabla \cdot D \nabla c + r$$

$$\frac{P_{A \rightarrow B}}{P_{B \rightarrow A}} = e^{-\Delta G_{AB} / (RT)}$$

$$\frac{dp_j(t)}{dt} = \sum_{i \neq j} Q_{ij} P_i - \sum_{i \neq j} Q_{ji} P_j$$



Daniel A. Beard

CAMBRIDGE

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*Medical College of Wisconsin*



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# Preface

Research, development, and design in bioengineering, biomedical engineering, biophysics, physiology, and related fields rely increasingly on mathematical modeling and computational simulation of biological systems. Simulation is required to analyze data, design experiments, develop new technology, and simply to attempt to understand the complexity inherent in biological systems.

This book focuses on practical implementation of techniques to study real biological systems. Indeed, whenever possible, specific applications are developed, starting with a study of the basic operation of the underlying biological, biochemical, or physiological system and, critically, the available data. It is hoped that this data-rich exposition will yield a practical text for engineering students and other readers interested primarily in earthy real-world applications such as analyzing data, estimating parameter values, etc. Thus for the examples developed here, important details of underlying biological systems are described along with a complete step-by-step development of model assumptions, the resulting equations, and (when necessary) computer code. As a result, readers have the opportunity, by working through the examples, to become truly proficient in *biosimulation*.

In this spirit of soup-to-nuts practicality, the book is organized around biological and engineering application areas rather than based on mathematical and computational techniques. Where specific mathematical or computational techniques can be conveniently and effectively separated from the exposition, they have been and can be found in the Appendices. Computer codes implemented in MATLAB® (The MathWorks, Natick, MA, USA) for all of the examples in the text can be found online at the URL <http://www.cambridge.org/biosim>.

I am particularly grateful to a number of individuals who provided critical feedback on the text, including Edmund Crampin, Peter Hunter, Muriel Mescam, Gary Raymond, Nic Smith, Matt Thompson, Kalyan Vinnakota, and Fan Wu. Andy Salmon graciously provided the data from his experiments presented in Section 2.2.2. Tom O'Hara provided some guidance on the model analyzed in Section 8.3. Jim Bassingthwaighe's guidance and advice over many years, as well as specific criticism of the text, are gratefully acknowledged.

Finally, I want to give special thanks to my colleagues Henry and Nicholas Beard for helping with the experiments described in Chapter 1.

# Extracts

“All is in flux.”

*Heraclitus (540–480 BCE)*

“This application of mathematics to natural phenomena is the aim of all science, because the expression of the laws of phenomena should always be mathematical.”

*Claude Bernard, Introduction a l'étude de la médecine expérimentale 1865 Flammarion, Paris  
(English translation from Noble, Exp. Physiol. 93: 16–26, 2008)*

“Of physiology from top to toe I sing.”

*Walt Whitman, Leaves of Grass, 1883*

“The human body is a chemical and physical problem, and these sciences must advance before we can conquer disease.”

*Henry A. Rowland, The Highest Aims of the Physicist, Address to the American  
Physical Society, 1899, published in Science 10: 825–833, 1899*

“We are seeing the cells of plants and animals more and more clearly as chemical factories, where the various products are manufactured in separate workshops. The enzymes act as the overseers. Our acquaintance with these most important agents of living things is constantly increasing. Even though we may still be a long way from our goal, we are approaching it step by step. Everything is justifying our hopes. We must never, therefore, let ourselves fall into the way of thinking ‘ignorabimus’ (‘We shall never know’), but must have every confidence that the day will dawn when even those processes of life which are still a puzzle today will cease to be inaccessible to us natural scientists.”

*Eduard Buchner, Nobel Lecture, 1907*

“To a physician or physiologist at the present day a man’s body is a machine, or rather a factory full of machines, all working harmoniously together for the good of the organism.”

*Ernest N. Starling, The Linacre Lecture on the Law of the Heart, 1915,  
published by Longmans, Green and Co., London, 1918*

“The mathematical box is a beautiful way of wrapping up a problem, but it will not hold the phenomena unless they have been caught in a logical box to begin with.”

*John R. Platt, Strong inference. Science, 146: 347–353, 1964*

“People who wish to analyze nature without using mathematics must settle for a reduced understanding.”

*Richard Feynman*

“[This book] is aimed at ‘non-believers’, that is to say the 90% or so of biochemistry students, and indeed of practicing biochemists, who place enzyme kinetics in the same category as Latin and cold showers, character-building perhaps, but otherwise to be forgotten as quickly as possible.”

*Paul C. Engel, Enzyme Kinetics: The Steady-State Approach 1977, Chapman & Hall, London*

“Why make models? To think (and calculate) logically about what components and interactions are important in a complex system.”

*James E. Bailey, Mathematical modeling and analysis in biochemical engineering: past accomplishments and future opportunities. Biotechnol. Prog., 14: p. 8–20, 1998*

“Without data, there is nothing to model; and without models, there is no source of deep predictive understanding.”

*James B. Bassingthwaite, The Physiome Project: The macroethics of engineering toward health. The Bridge, 32: 24–29, 2002*

“Over the last half century, we have proceeded by breaking living systems down into their smallest components, the individual genes and molecules. Humpty Dumpty has been smashed into billions of fragments . . . Can we put Humpty Dumpty back together again?”

*Denis Noble, The Music of Life: Biology beyond the Genome. 2006, Oxford, New York*



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# 1 Introduction to simulation of biological systems

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## Overview

This chapter is built around analyzing a real data set obtained from a real biological system to illustrate several complementary approaches to simulation and analysis. The particular system studied (a home aquarium) is a well-mixed chemical reactor. Or, more accurately, the system studied is treated as a well-mixed chemical reactor, a basic modeling paradigm that will appear again and again in this book.

Here, we look at this single physical system from several different perspectives (that is, under different sets of underlying modeling assumptions) with the aim of motivating the reader to undertake the study of the rest of this book. The aim is not to overwhelm the reader with mathematical details that can be found in later chapters. Therefore let us clearly state at the outset: it is not expected or required that the reader follow every detail of the examples illustrated here. Instead, we invite the reader to focus on the basic assumptions underlying the methods applied, and to compare and contrast the results that are obtained based on these different approaches. Proceeding this way, it is hoped that the reader may gain an appreciation of the breath of the field. Furthermore, it is hoped that this appreciation will continue to grow with a study of the rest of this book and beyond.

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## 1.1 Modeling approaches

The number of different approaches to simulating biosystems behavior is perhaps greater than the number of biological systems. The number is at least large enough that a finite and complete list cannot be constructed. Simulation methods may be classified according to the physical systems simulated (for example, cellular metabolism, whole-body drug distribution, or ecological network dynamics), the sets of assumptions used to build a simulation (for example, rapid mixing versus spatial inhomogeneity in chemical reaction systems), or the mathematical/computational formulation of the simulation (for example, systems of ordinary differential equations versus statistical inference networks for describing

regulation of gene transcription). A glance at the table of contents reveals that most of this book is organized by biological system or application area. (Modeling assumptions and relevant computational techniques are introduced as necessary.)

These biological systems can (and will!) be studied by applying a variety of sets of assumptions and associated computational methods. Doing this, we will see that the methodology applied to a given system depends strictly on what one thinks one knows about the system in advance, and what one wishes to discover through computational analysis. In the following introductory example we will see that what we can learn (for example, what variables and what parameters we can estimate) depends on the prior knowledge built into a model, including (but not limited to) what data are available for a given system.

## 1.2 An introductory example: biochemistry of a home aquarium

As our first exemplar modeling study, let us analyze the buildup and reaction of waste materials in a home aquarium, a system that may be familiar to some readers. Ammonia ( $\text{NH}_3$ ), which is toxic to fish, is excreted from fish as a waste product and produced through decomposition of organic matter. In a well-functioning aquarium, nitrifying bacteria in the aquarium filter oxidize ammonia to nitrite ( $\text{NO}_2^-$ ) and oxidize the nitrite to nitrate ( $\text{NO}_3^-$ ). Of these three nitrogen-containing compounds, nitrate is by far the least toxic to fish.

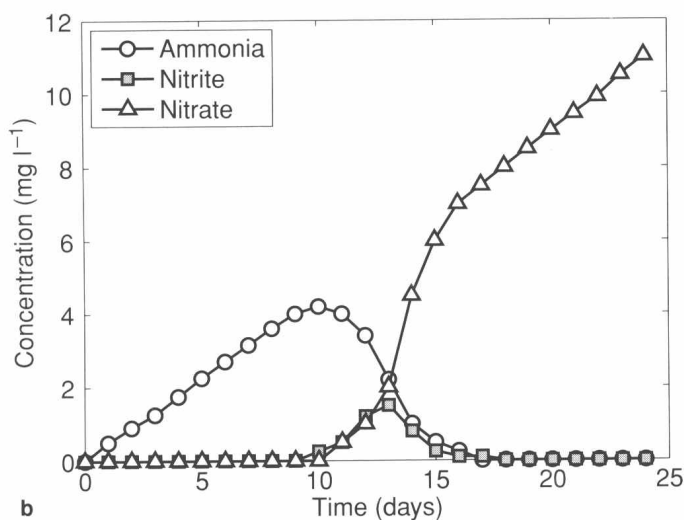
When one sets up a new aquarium, populations of nitrifying bacteria are yet to be established, and concentrations of toxic compounds can temporarily build up. Figure 1.1 plots data collected by the author from his own aquarium following the addition of fish into a previously uninhabited new tank. Here we see that ammonia concentration tends to build up over the first week or more. Once significant populations of bacteria that convert  $\text{NH}_3$  to  $\text{NO}_2^-$  appear, the ammonia declines while the nitrite level increases. Nitrite concentration eventually declines as nitrate begins to appear.<sup>1</sup>

We wish to understand how these three concentrations are related kinetically. To simplify the notation, we introduce the definitions  $x_1 = [\text{ammonia}]$ ,  $x_2 = [\text{nitrite}]$ ,  $x_3 = [\text{nitrate}]$  for the concentration variables. As already described, the expected sequence of reaction in this system is  $x_1 \rightarrow x_2 \rightarrow x_3$ . In fact, that sequence is apparent from the data illustrated in the figure. Ammonia ( $x_1$ ) peaks around day 10, followed by nitrite ( $x_2$ ) around day 13. Nitrate concentration ( $x_3$ ) really picks up following the peak in nitrite, and continues to steadily increase.

<sup>1</sup> Ammonia, nitrite, and nitrate exist in aqueous solution in a number of rapidly interconverting forms. For example, at low pH  $\text{NH}_3$  is largely protonated to form the ammonium ion  $\text{NH}_4^+$ . Here, the terms ammonia, nitrite, and nitrate are understood to include all such rapidly converting species of these reactants.



a



b

**Figure 1.1**

A home aquarium. The plot in panel (b) shows ammonia, nitrite, and nitrate concentration versus time in a home aquarium. Concentrations are given in units of mg of nitrogen per liter ( $\text{mg l}^{-1}$ ).

Yet in addition to the reaction sequence, is it possible to obtain additional quantitative information from these data? To do so, let us construct a series of simple models and see what we can find.

### 1.2.1 First model: a nonmechanistic analysis

In the first model we would like to introduce the minimum number of assumptions that allow us to explain the observed data. The idea is to construct a general set of

governing differential equations for  $x_1$ ,  $x_2$ , and  $x_3$  based only on the assumption of mass conservation. Conservation laws are a universal tool for developing models of many systems, including practically all of the examples studied in this book. Here we assume that the volume of the system,  $V$ , remains constant. So the rate of change of mass of a substance,  $\frac{d(cV)}{dt}$ , is equal to  $V \frac{dc}{dt}$ . In this case, the general differential equation for concentration of a reactant is

$$V \frac{dc}{dt} = \left( \begin{array}{c} \text{rate of production or} \\ \text{input measured in units} \\ \text{of mass per unit time} \end{array} \right) - \left( \begin{array}{c} \text{rate of loss} \\ \text{measured in units of} \\ \text{mass per unit time} \end{array} \right) \quad (1.1)$$

or

$$\frac{dc}{dt} = \left( \begin{array}{c} \text{rate of production or} \\ \text{input measured in units} \\ \text{of mass per unit time} \\ \text{per unit volume} \end{array} \right) - \left( \begin{array}{c} \text{rate of loss} \\ \text{measured in units of} \\ \text{mass per unit time} \\ \text{per unit volume} \end{array} \right). \quad (1.2)$$

Applying this general form to the variables  $x_1$ ,  $x_2$ , and  $x_3$  gives

$$\begin{aligned} \frac{dx_1}{dt} &= k(t) - r_{12}(t) \\ \frac{dx_2}{dt} &= r_{12}(t) - r_{23}(t) \\ \frac{dx_3}{dt} &= r_{23}(t), \end{aligned} \quad (1.3)$$

where  $k(t)$  is the rate of ammonia ( $x_1$ ) production, and  $r_{12}(t)$  and  $r_{23}(t)$  are the rates of conversion from ammonia to nitrite and from nitrite to nitrate, respectively. The first equation states that the rate of change of  $x_1$  is equal to the rate of production minus the rate of degradation. Similar statements of mass conservation follow for  $dx_2/dt$  and  $dx_3/dt$ . Since no processes degrading nitrate are considered, there is no degradation term in the  $dx_3/dt$  equation. Because  $k(t)$ ,  $r_{12}(t)$ , and  $r_{23}(t)$  are (so far) assumed to be arbitrary functions, we have (so far) not introduced any assumptions about the rules governing the behavior of these functions. The names and definitions of the model variables are listed in the table below.

Variable	Units	Description
$x_1$	$\text{mg l}^{-1}$	concentration of ammonia
$x_2$	$\text{mg l}^{-1}$	concentration of nitrite
$x_3$	$\text{mg l}^{-1}$	concentration of nitrate
$k$	$\text{mg l}^{-1} \text{ day}^{-1}$	rate of ammonia production
$r_{12}$	$\text{mg l}^{-1} \text{ day}^{-1}$	rate of nitrite production from ammonia
$r_{23}$	$\text{mg l}^{-1} \text{ day}^{-1}$	rate of nitrate production from nitrite

We call this model “nonmechanistic” because it does not invoke any biochemical/biophysical mechanisms to describe the rates of conversion  $r_{12}(t)$  and  $r_{23}(t)$ , or the rate of production  $k(t)$ . Instead these rates are all allowed to be arbitrary functions.

So what can we do with this simple general model? One useful thing we can do is analyze the data using the model to estimate  $r_{12}(t)$ ,  $r_{23}(t)$ , and  $k$  and test the model assumptions. From Eq. (1.3), we have  $r_{23}(t) = dx_3/dt$ , which can be numerically approximated using a finite difference approximation

$$\hat{r}_{23}(t) \approx \frac{x_3(t + \Delta t) - x_3(t - \Delta t)}{2\Delta t}. \quad (1.4)$$

Here  $\Delta t$  is the discrete time step over which the data in Figure 1.1 are sampled. (I took one measurement per day, so  $\Delta t = 1$  day.) Equation (1.4) is the “central-difference” approximation for the derivative of  $x_3$  with respect to time.<sup>2</sup> Here we use the notation  $\hat{r}_{23}$  to denote the approximation (from the data) of  $r_{23}$ . Next, given our approximation of  $r_{23}(t)$ , we can approximate  $r_{12}(t)$ :

$$\hat{r}_{12}(t) = \frac{dx_2}{dt} + r_{23}(t) \approx \frac{x_2(t + \Delta t) - x_2(t - \Delta t)}{2\Delta t} + \hat{r}_{23}(t). \quad (1.5)$$

Similarly, we can approximate  $k$  as a function of time

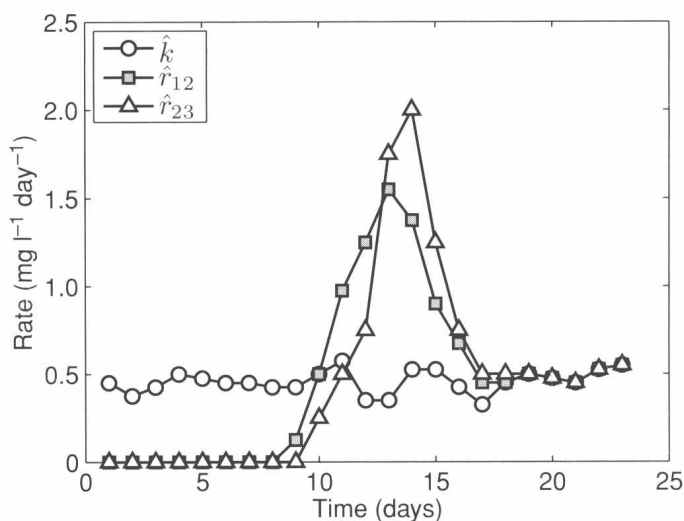
$$\hat{k}(t) = \frac{dx_1}{dt} + r_{12}(t) \approx \frac{x_1(t + \Delta t) - x_1(t - \Delta t)}{2\Delta t} + \hat{r}_{12}(t). \quad (1.6)$$

Values of  $\hat{k}(t)$ ,  $\hat{r}_{12}(t)$ , and  $\hat{r}_{23}(t)$  computed from the data in Figure 1.1 are plotted in Figure 1.2.<sup>3</sup> From these estimated rates we learn a number of things about this system not immediately apparent from a simple inspection of the raw data. First, we can see that the rate of ammonia production ( $\hat{k}(t)$ ) is estimated to be approximately constant. Moreover, this analysis provides an estimate of the constant  $k$ , approximately  $0.4$  to  $0.5 \text{ mg l}^{-1} \text{ day}^{-1}$ . This observation is perhaps not unexpected, because the number and size of the fish remained approximately constant over the course of the experiment, as did the amount of food introduced per day. Therefore we might have expected the rate of ammonia production to be nearly constant. Second, the analysis reveals that nitrite production ( $\hat{r}_{12}(t)$ ) peaks near day 13 while nitrate production peaks shortly after, around day 14. Towards the end of the experiment, all of the reaction rates converge to equal approximately  $0.5 \text{ mg l}^{-1} \text{ day}^{-1}$ . Finally, we note that the estimated rates  $\hat{k}(t)$ ,  $\hat{r}_{12}(t)$ , and  $\hat{r}_{23}(t)$  remain positive for the duration of the experiment. This observation makes sense, because under normal conditions neither of the nitrification reactions is expected

<sup>2</sup> Discrete approximations of derivatives are reviewed in Section 9.1 in the Appendices.

<sup>3</sup> Computer codes (implemented in MATLAB) for this and all of the examples in this book can be found online at the URL <http://www.cambridge.org/biosim>.



**Figure 1.2**

Values of  $\hat{k}(t)$ ,  $\hat{r}_{12}(t)$ , and  $\hat{r}_{23}(t)$  estimated from Eqs (1.4)–(1.6) and the data in Figure 1.1. These rates are expressed in units of mass of nitrogen per unit volume per unit time:  $\text{mg l}^{-1} \text{ day}^{-1}$ .

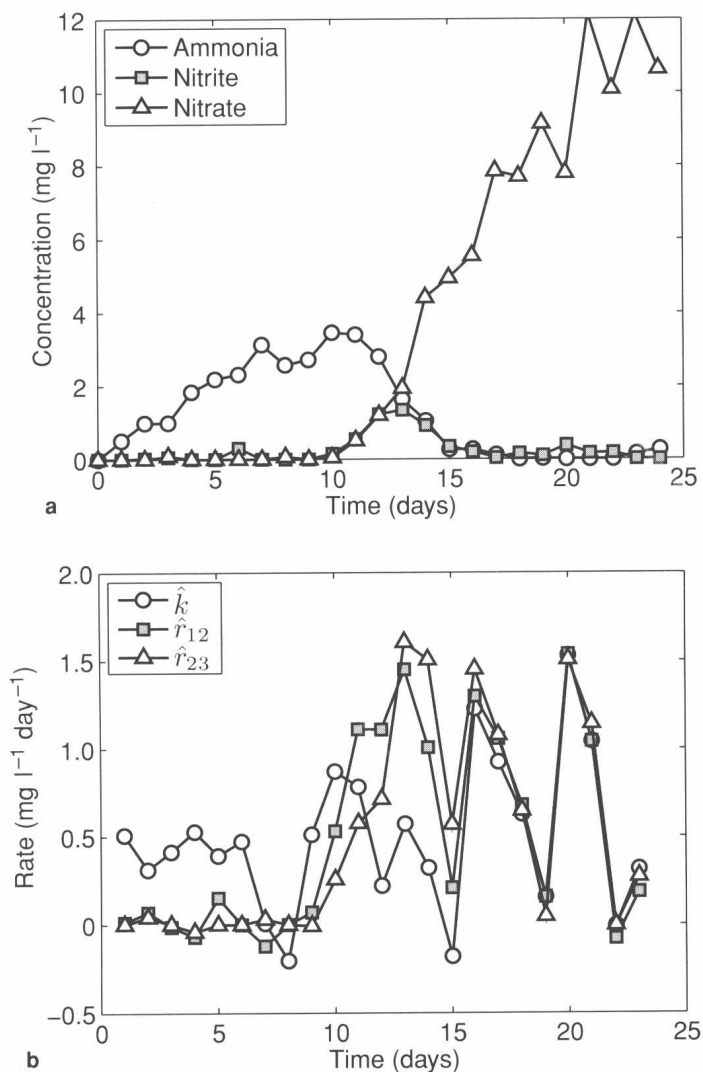
to proceed in the reverse direction. Thus the result that  $\hat{r}_{12}(t)$  and  $\hat{r}_{23}(t)$  remain positive provides a useful check of the physical realism of the model.

To summarize, analyzing the data of Figure 1.1 using the simple model of Eq. (1.3), which invokes no more serious assumption than conservation of mass, provides quantitative estimates of a number of variables that are not directly measured.

## 1.2.2 Nonmechanistic analysis with noise

The preceding analysis was applied to a relatively noise-free data set, yielding reasonable (and smooth) numerical estimates for the derivatives in Eqs (1.4)–(1.6). However, differentiation has the unfortunate side effect of tending to magnify noise. And since significant measurement noise is often associated with real-world biological signals, analyses that require the estimation of derivatives of biological data are often seriously confounded by noise.<sup>4</sup>

<sup>4</sup> The aquarium experiment studied here cannot be regarded as a precisely controlled study. The original data set of Figure 1.1 was collected by the author using a simple consumer kit, with which the concentrations are estimated by visual comparison of the fluid in a test-tube assay with a color chart, possibly introducing bias. Although dilutions and replicates were performed as appropriate, there is a human psychological component to interpreting these assays. Furthermore, because the nitrate assay used is relatively insensitive over the reported concentration range, data were obtained by a combination of interpolation, assuming a constant total nitrate production rate, and colorimetric assay. Given the potential for bias, the reader is encouraged to conduct his or her own experiments in his or her own home laboratory!

**Figure 1.3**

Analysis of noisy aquarium data. In panel (a) the data of Figure 1.1 are reproduced with added noise. The analysis of the previous section is reproduced in panel (b), with  $\hat{k}(t)$ ,  $\hat{r}_{12}(t)$ , and  $\hat{r}_{23}(t)$  computed from Eqs (1.4)–(1.6) applied to the noisy data from panel (a).

To illustrate this problem, and to explore some ideas of how to deal with it, we can add some noise to our aquarium data. Figure 1.3(a) shows the same data as those of Figure 1.1, with a relatively small amount of noise added. We can see that the basic trends in the data remain the same, but this data set is less smooth than the previous one.

The values of  $\hat{k}(t)$ ,  $\hat{r}_{12}(t)$ , and  $\hat{r}_{23}(t)$ , computed from Eqs (1.4)–(1.6) for these data are plotted in Figure 1.3(b). Here we can see the consequence of differentiating