MATHEMATICAL MODELING IN BIOTECHNOLOGY

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Contents

Summary

Mathematical models are important tools in the optimization of the performance of biotechnological processes. This article shows how modeling is applied to fermentation and enzyme-based processes. It begins by analyzing the structure of mathematical models, showing how they contain state variables, independent variables, operating variables and parameters. A case study, in which differential equations are derived to describe the operation of a simple enzyme reactor, is presented to illustrate these concepts. This provides the basis for a discussion of the various approaches that can be taken to modeling, such as empirical versus mechanistic approaches, or structured versus unstructured approaches. The article then gives an overview of the general steps that are required in the development and use of a mathematical model, from the initial definition of the aims of the modeling work, to the final application of the model to achieve those aims. Two further case studies are presented to illustrate these basic concepts. Firstly, a mathematical model is developed for an enzyme reactor consisting of a reservoir containing a substrate solution which is circulated through a column with a bed containing immobilized enzyme. The use of the model to estimate kinetic constants of the enzyme is demonstrated. Secondly, a structured metabolic model developed in 1984 for the growth of a single cell of *Esherichia coli* is discussed. Finally, the future contributions that are likely to be made by models in biotechnology are evaluated.

1. What Can Mathematical Models Do for Biotechnological Processes?

Mathematical models are tools that we can use to describe the past performance and predict the future performance of biotechnological processes. They can be applied to processes operating at many different levels, from the action of an enzyme within a cell, to the growth of that cell within a commercial scale bioreactor. Mathematical models can be powerful tools in both fundamental research and applied research and development. For example, some models contribute to our understanding of how cells function, while other models allow us to use laboratory and pilot-scale data to make predictions about how a commercial scale bioreactor must be designed and operated in order to give optimal performance.

Although many different types of biotechnological systems and processes can be modeled, such as the operation of metabolic pathways within a cell, the expression of genes within a cell, the death of cells during a sterilization process, the growth of cells in a bioreactor and the action of enzymes, to name a few, this article focuses specifically

on the modeling of fermentation and enzymatic processes carried out in bioreactors. Furthermore, although mathematical models can be of various different forms, this article concentrates on models consisting of differential equations.

Differential equations describe, in a simplified manner, how the key physical and biological phenomena operate. Under some conditions these differential equations can be either integrated analytically or simplified to give algebraic equations, but this is very often not the case. Figure 1 gives a simplified illustration of how a model consisting of differential equations might be applied to a fermentation process.

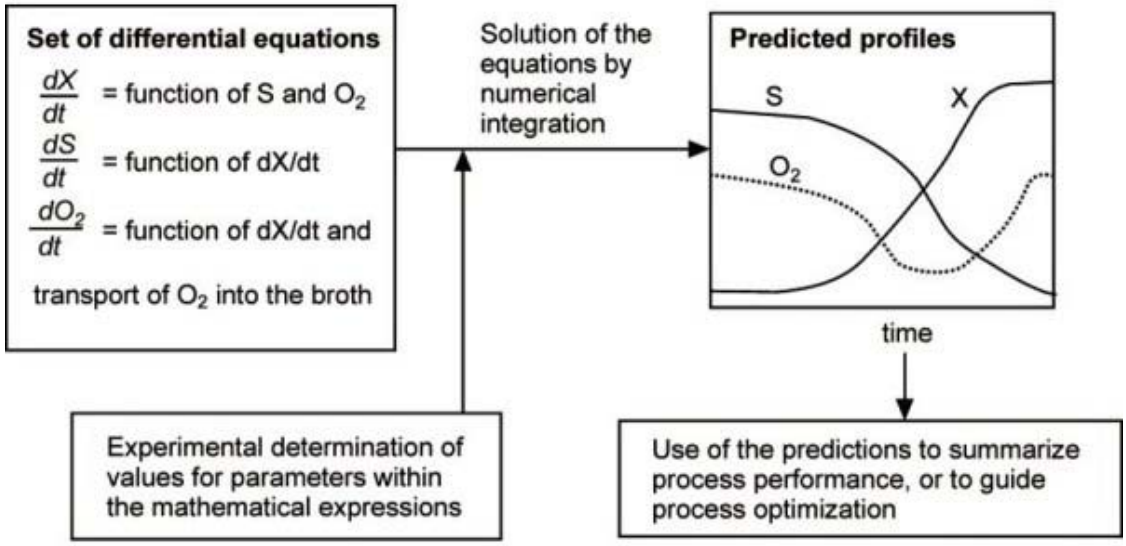

Figure 1: The application of a differential model to a fermentation process

One equation may include a description of how the rate of growth of the biomass depends on the oxygen and substrate concentrations in the fermentation broth, another equation may include a description of how substrate is consumed during growth, while still another equation may describe how the rate of transfer of oxygen from the gas bubbles into the fermentation broth depends on the relative oxygen concentrations in the two phases. These differential equations describe the rates of change of biomass, oxygen and substrate concentrations but not the actual values of these variables. The equations are then solved by numerical integration.

The solution consists of predicted profiles, plotted against time, for the biomass, oxygen and substrate concentrations. These predictions can be utilized in various ways. Typically, the predictions will first be compared against experimental results. If they describe the results well, then the model can be used to explore the effects on the growth of the biomass of the initial substrate concentration and the aeration rate with the aim of identifying the conditions for optimal growth. Fermentation will be done using the best values identified by the model to confirm that the bioreactor does in fact perform better under these conditions.

The importance of mathematical models can be seen by imagining a world without them, in which we were limited to tabulating or graphing measurements made during

fermentations and were unable use equations to describe the processes occurring. With this limitation it would be difficult to summarize the large array of data in a manner that would allow us to obtain a deep understanding of the complex interactions between the microorganism, the environmental conditions and the transport phenomena occurring within the bioreactor. In contrast, mathematical models can summarize quite complex behavior in a relatively small number of equations.

In effect, these models represent hypotheses as to how these biological and physical phenomena interact to control the process. If the predictions agree with experimental data, it is quite possible that the phenomena described in the model are actually the key phenomena controlling process performance.

Models can be used within sophisticated on-line control schemes that measure the current state of the process and use the model to predict how aeration rates and substrate feed rates should be changed to increase the rates of growth and product formation.

One of the most useful features of models is that they can make predictions about behavior of the system outside of the range of conditions for which they were originally developed. Of course this does not mean that the biological system will behave as predicted under these new circumstances, but this does not negate the usefulness of the model as a tool in guiding research and development. A combined modeling-experimental program has a better chance of improving the process, and doing so more rapidly, than a purely experimental program.

The sections that follow first describe the components of mathematical models and present a simple case study showing the construction of equations. This provides background for a description of the general steps of a modeling project. This description shows how the original objective of the modeling project guides decisions about the number of equations that will be used and what structure they will have, and also how a modeling project requires experimental work to determine parameters and validate the model.

However, this description does not contain sufficient information for a reader who is new to modeling to learn how to construct a model of their own fermentation system. The article ends with two modeling case studies and an evaluation of the future prospects of modeling in biotechnology.

2. Overview and General Principles of Mathematical Modeling Biotechnological Processes

2.1. Elements of a Mathematical Model

A differential model consists of a set of differential equations involving state variables, independent variables, operating variables and parameters. These will be explained below in the context of a relatively simple biological reactor, an enzyme reactor, in which an enzyme is used to convert a substrate into a desired product (Figure 2).

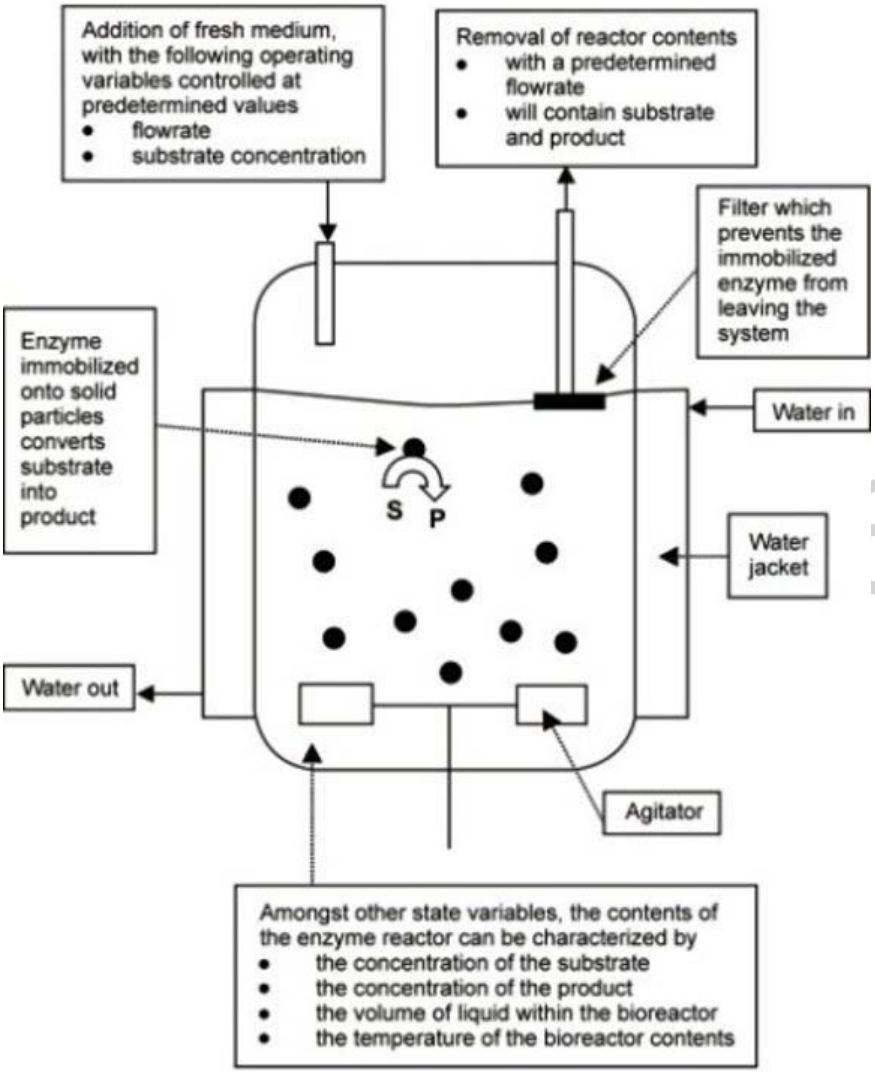

Figure 2: Diagram of an enzyme reactor, showing several of the important state and operating variables within the system

A state variable is a variable that represents the state of the system. In the enzyme reactor there are many state variables, such as the enzyme concentration, the substrate concentration, the product concentration within the aqueous phase, the volume of the aqueous phase, and the temperature and the pH within the reactor. The combination of the state variables at an instant gives a picture of the state of the system at that instant. A model of an enzyme bioreactor will attempt to describe how one or more of these state variables change with time.

Independent variables are those variables that do not depend on the processes in the system. In fact, it is the opposite which is true. The state of the system varies as the independent variables vary. The independent variables that appear in bioreactor models most frequently are time and one or more of the spatial dimensions (Figure 3). For example, in a well-mixed enzyme reactor operated in batch mode, concentrations of substrate and product will change with time. For a bioreactor operated in plug-flow mode, it is possible to have a steady state in which the system does not change with

time, but in which the concentrations of substrate and product are functions of the position in the reactor. In batch systems that are not well-mixed, the concentrations will be functions of both time and position. Note that steady-states are also possible in which the state variables are independent of any independent variable. For example, in a well-mixed fermentation operated in continuous mode, concentrations of substrate, biomass and product remain steady with time and are the same at all positions within the bioreactor.

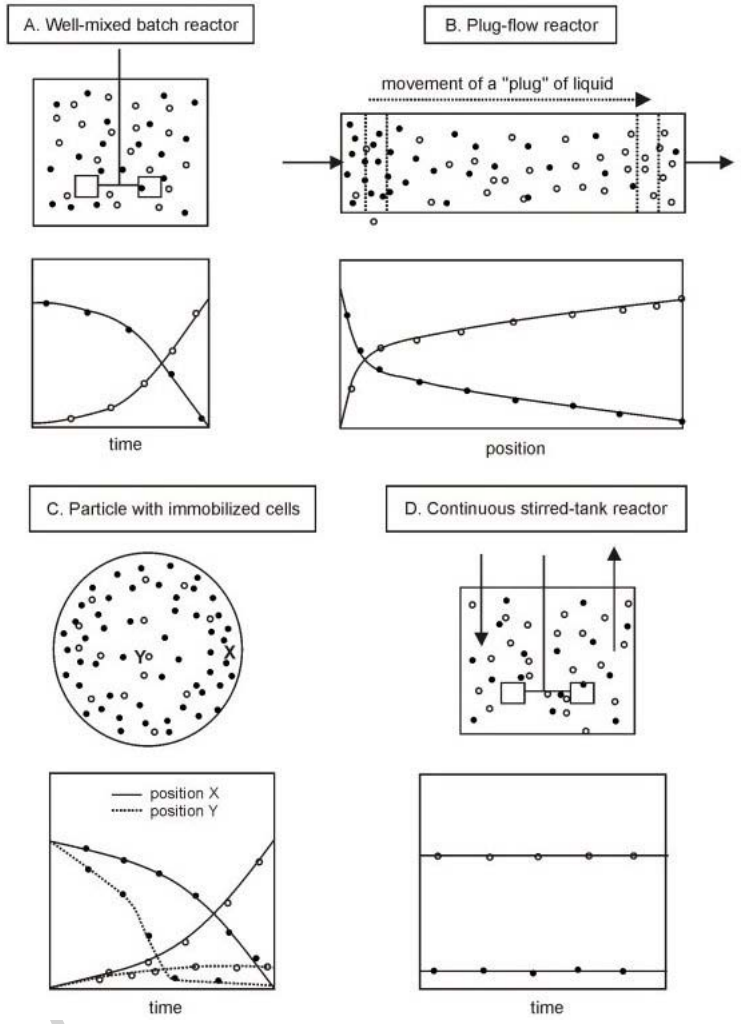

Figure 3: The influence of bioreactor operation on whether the state variables depend on position or time or both. On the graphs the vertical axis represents concentration. (•) Substrate (○) Product;

A) A batch process in a well-mixed bioreactor. State variables are a function of time but not of position; B) A plug flow bioreactor at steady state. The spatial profiles do not change with time. The dotted regions and the dotted arrow demonstrate the concept of plug flow. If the dotted region on the left can be thought of as a plug of liquid, then this plug travels as a whole down the bioreactor. Note that plug flow is an ideal situation. Often the fluid flows faster along the central axis of the reactor than it does nearer to the walls. C) A particle within a bioreactor, within which either cells or enzymes are

immobilized. State variables are functions of both time and position. D) A continuous stirred-tank bioreactor, operated at steady state. State variables are independent of both time and position.

Operating variables represent various inputs into the system that can be independently controlled. These can be used to influence the performance of the system. For the enzyme reactor operated in continuous mode, some of the operating variables are the stirrer speed, the temperature of the water in the thermal jacket surrounding the reactor, the rate of feed of substrate to the process, and the rate at which liquid is removed from the process.

Parameters are intrinsic properties of the system or parts of the system. Several parameters of the enzyme bioreactor system include the heat capacity, density and viscosity of the aqueous phase, the catalytic rate and affinity constant of the enzyme, and the coefficient for transfer of heat across the wall of the bioreactor. These parameters may be essentially constant under the operating conditions of the bioreactor, or alternatively, they may depend on the state variables. For example, the catalytic rate of an enzyme is typically significantly affected by temperature changes of only a few degrees.

A differential model typically expresses how several of the state variables vary with variations in one or more independent variables (i.e. they express changes in state variables with time or position or both), expressing these changes as mathematical equations involving state variables, independent variables, operating variables and parameters.

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Biographical Sketches

Professor Mata-Alvarez graduated in Chemistry at the University of Barcelona and obtained a Master of Environmental Sciences at the EOI in Madrid. His PhD thesis was in the area of methods of optimization in Chemical Engineering. He became a Professor of the Chemical Engineering Department of the University of Barcelona in 1985 and has spent periods in the position of Visiting Professor at the University of Venice (Italy), at the University of California (Davis) and at the University of Gent. He has acquired industrial experience in the Chemical Engineering Department at the Headquarters of Hoechst in Germany and he has also spent a full year in AGBAR (a society devoted to processing water for drinking purposes). He has been working in environmental related research subjects, especially biological treatment, for the last 20 years. He is the author of several books and more than 130 scientific papers about this subject in Spanish and International journals, and he is also author or co-author of more than 170 communications in different Symposia and Congresses. He has also been involved in various scientific committees of Spanish and International congresses. He is a Spanish representative within the Bioreactor Performance section of the European Federation of Chemical Engineering. He is a member of various international organizations, including: International Water Association (IWA), International Organization of Biotechnology and Bioengineering (IOBB), Water Environment Federation (WEF) and International Solid Waste and Public Cleansing Association (ISWA).

David Mitchell obtained a Bachelor of Science with honors in Microbiology, and a PhD in Biotechnology, both from The University of Queensland, in Brisbane, Australia. After 9 years as a lecturer in the Department of Chemical Engineering at The University of Queensland, he migrated to Brazil and is currently teaching within the Biochemistry and Molecular Biology Department of the Federal University of Paraná. He has published extensively in the area of solid-state fermentation, where, for the past several years, he has been using mathematical modeling as a tool to understand how microbial growth in solid-state bioreactors is controlled by diffusion processes within particles and heat and mass transfer phenomena within the substrate bed.