Cybernetic Modeling for Bioreaction Engineering

Uniquely focusing on dynamic modeling, this volume incorporates metabolic regulation as a survival mechanism for cells by driving metabolism through optimal investment of its resources for control of enzyme synthesis and activity. Consequently, the models have a proven record of describing various uptake patterns of mixed carbon substrates that have become significant in modern applications of biomass for the production of bioenergy. The models accurately describe dynamic behavior of microbes in nutrient environments with mixtures of complementary substrates, such as carbon and nitrogen. Modeling of large metabolic networks (including prospects for extension to genome scale) is enabled by lumped hybrid cybernetic models with an unparalleled capacity to predict dynamic behavior of knockout strains. This is an invaluable, must-have reference for bioresearchers and practicing engineers.

Professor Doraiswami Ramkrishna is the Harry Creighton Peffer Distinguished Professor of Chemical Engineering at Purdue University. He pioneered the development of dynamic metabolic modeling and has been active in the area for over thirty years. He is a member of the National Academy of Engineering, coined the term "cybernetic modeling," and has authored several academic books.

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To
our students in the cybernetic group

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Preface

This book is an outgrowth of nearly three decades of work by our research group and is therefore rightly dedicated to the many students responsible for developing the ideas of modeling microbial systems that have led to its current state of the art.

It began modestly on viewing biomass as an entity devoid of structure except for "key" enzymes that were responsible for the uptake of a mixture of external carbon substrates such as glucose and xylose. The goal was to examine whether it was possible to describe the phenomenon of "diauxic" growth observed by Monod in 1942 with bacteria, which consisted in the preferred utilization of glucose, and utilizing the substrate xylose only after all glucose had been nearly consumed. Monod attributed this phenomenon to metabolic regulation by which enzymes for the uptake of glucose were preferentially synthesized while those for xylose were not. When glucose dropped to low enough levels, expression of enzymes ensued for the uptake of xylose upon which growth on xylose and the glucose that remained occurred together. Monod's experiments with numerous substrate pairs showed that diauxic behavior invariably occurred with preferential utilization of the substrate that supported a higher growth rate. Building on this clue, we were led to postulate that the organism must make frugal use of its resources for enzyme synthesis so that the resulting growth rate is maximized. Indeed, known molecular details of this regulatory phenomenon could have enabled a more "mechanistic" model, but the temptation was strong to seek a description that had the potential to take a more comprehensive account of regulatory processes at large. Many articles had appeared on how living systems, even microorganisms as products of evolution, must be viewed as capable of responding to their environment calculated to promote their survival. The implication was the existence of a sophisticated machinery in living systems that may have evolved as a "genetic" program, which could craft and execute a survival response to the organism's environment. If describing the execution was forbiddingly complex, focusing on the strategy alone appeared to make for an attractive alternative for accommodating metabolic regulation. Yet another fundamental issue was the inevitable role of teleology (or more appropriately "teleonomy") without which explanations of biological phenomena would be drab and devoid of the character of associating events with sustaining life; to entertain regulatory phenomena without an underlying purpose seemed in contradiction with the use of the term "regulation." These observations added up to viewing the cell as a cybernetic system, the term "cybernetic" arising from the Greek word $\chi\upsilon\beta\epsilon\rho\nu\epsilon\tau\eta\sigma$ or *cybernetes* meaning "steersman"; in other words, cell response is under navigation toward a survival goal. This navigation is

accomplished through a molecular infrastructure whose description and function are unessential to the theory.

With the foregoing background, one of us (DR) discussed in an invited lecture at the American Chemical Society some preliminary thoughts in 1982, reproduced in Ramkrishna (1983), toward developing a mathematical framework for the scenario just outlined. This heralded an effort toward the development of a theory that has evolved over nearly three decades piloted by Dhurjati's doctoral dissertation (1982) in which the growth rate of the organism was maximized over a period specified by a small amount of residual substrate. This optimization was to be accomplished by investment of a fixed amount of resource for the synthesis of enzymes needed to metabolize two different carbon sources. This approach had two difficulties. The computational demands of the resulting singular control problem was incompatible with the goal of extracting the dynamics of the growth process. The finite time horizon for the optimization was felt to be an unsatisfactory feature. A much simpler theory based on the heuristics of maximizing the instantaneous growth rate (zero time horizon) followed in Kompala's dissertation (1984). The theory successfully described diauxic behavior for several substrate pairs and even accommodated interim lags between the sequential use of substrates. However, growth at low substrate levels failed to connect with experiments so that the chemostat scenario, especially at low dilution rates, was quite out of accord with predictions. The doctoral dissertations of Turner and Baloo sought to correct this situation by including maintenance effects–the implication being the preference for maintenance over growth in the famine situations of low substrate levels. The models handled transients in batch, fed batch, and continuous cultures with mixed substrates. Alexander's thesis (1990) followed with a detailed, structured model that was successful with applications to product formation.

While each of the foregoing dissertations contributed important elements to the growth of the framework, Straight (1991) was the first to be concerned about addressing metabolic networks. He sought to decompose the network into segments that were linear, converging or diverging, and cycles. Varying objectives assigned to individual units produced different controls that Straight used to describe metabolic performance with complementary substrates. While the mathematical treatment of optimality was akin to that in Kompala's work, Straight clearly produced a generation of cybernetic models distinct from its predecessors with intriguing success.

Kompala's success with prediction of the diauxic pattern raised concerns about how simultaneous consumption of mixed substrates (such as organic acids) could be addressed by cybernetic models. Narang (1994) approached this by performing systematic experiments with mixed substrates from which it became evident that uptake patterns of mixed substrates could be quite complex. Ramakrishna (1996), taking a cue from Straight's work, formulated a cybernetic model that used a simple network in which growth precursors were created for biomass synthesis from breakdown of the different substrates. The uptake pattern that prevailed at any instant was that which allowed the synthesis of precursors ensuring maximum growth rate. Sequential and simultaneous uptake patterns were predicted by the model under the conditions in which they were observed to occur. Straight (1991) provided considerable insight into