Dynamic modeling and analysis of oscillatory bioreactors

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DYNAMIC MODELING AND ANALYSIS OF OSCILLATORY BIOREACTORS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Chemical Engineering

by
Yongchun Zhang
B.S. Zhejiang University, China, 1995
M.S. Louisiana State University, 1999
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To my wife, parents, and family.
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Abstract

Dynamic modeling of bioreactors is a challenging problem. The complexity of first principle models also make model validation and analysis very difficult and model-based controller design practically intractable. This thesis has focused on finding an effective tool for model dynamic analysis, construction of low-dimension model and simple and effective controller design.

The validity of a biochemical reactor model often is evaluated by comparing transient responses to experimental data. Dynamic simulation can be rather inefficient and ineffective for analyzing bioreactor model. Bifurcation analysis is found to be a powerful tool for obtaining a more efficient and complete characterization of the model behavior. Dynamic behaviors of three low-dimension continuous bioreactor models consisting of a small number of ordinary differential equations are investigated. Several important features, as well as potential limitations, that are difficult to ascertain via dynamic simulation are disclosed through the bifurcation analysis. Bifurcation analysis is also successfully used for analysis and validation of more complex population balance models for yeast cultures.

*Saccharomyces cerevisiae* exhibits sustained oscillations over a wide range of operating conditions when produced in a continuous bioreactor. Transient cell population balance models consist of nonlinear partial differential-integro equations. An accurate discretized approximation which typically requires a large number of
nonlinear ordinary differential equations is not well suited for dynamic analysis and controller design purpose. Proper orthogonal decomposition is used to construct nonlinear reduced-order models from spatiotemporal data sets obtained via simulations of an accurate discretized yeast cell population model. The short-term and long-term behaviors of the reduced-order models are evaluated by comparison to the full-order model. Dynamic simulation and bifurcation analysis results demonstrate that reduced-order models with a comparatively small number of differential equations yield accurate predictions over a wide range of operating conditions.

Feedback linearizing control of the yeast bioreactor is also studied. The controller design is based on a low dimensional moment representation of the PBE model. Satisfactory oscillation attenuation results have been achieved. The performance of nonlinear controllers using different input/output variable pairings is also investigated.
Chapter 1

Introduction

1.1 Overview

Biochemical reactors are inherently nonlinear and very complex dynamic systems. The intracellular and extracellular environments are comprised of many chemical components and each cell has unique properties. Dynamic modeling of such systems is quite challenging. A rigorous mechanistic model for such a system usually consists coupled sets of ordinary differential and partial differential equations [15, 22, 76]. In addition to being difficult to formulate, these models are not amenable to systematic analysis due to their complexity. Simplified models can be developed by various abstracting approaches [11, 14, 21, 35, 42, 69]. Most of these models focus on the characterization of physical dynamic behaviors and are usually validated by comparing simulation results to experimental data. It is clearly of great interest to seek a more efficient and effective method for model validation and discrimination between controversial candidate models.

Baker’s yeast (Saccharomyces cerevisiae) has drawn special interest in dynamic modeling. Experimentally observed dynamic behaviors such as autonomous sustained oscillation and multiple stable solutions under same operating conditions has been a challenge for modeling researchers and the underlying causes are still contro-
versial and current topic of study. Notably, due to the essential segregated nature of the cell population, most models in the literature follow the population balance modeling framework.

A series of continuous research work has been carried out on the modeling and control of yeast bioreactors in LSU chemical engineering department [35, 59, 81, 89]. This research attempts to perform a comprehensive study of the modeling, dynamic analysis and nonlinear control of the yeast bioreactor based on population balance models. The research focuses on the following tasks:

- Use of bifurcation analysis as a model validation and discrimination tool for bioreactor systems.
- Development of reduced order models from the population balance models.
- Development of simple and effective nonlinear control techniques for yeast bioreactors.

The following sections in this chapter will present a brief introduction on the background knowledges for yeast culture dynamics and modeling, dynamic analysis and nonlinear model order reduction techniques. Chapter 2-4 will focus on each aspect of the above objectives. A summary of the work and recommendation for future research is presented in Chapter 5.
1.2 Yeast Culture Dynamics and Modeling

*S. cerevisiae* is an important microorganism in many industries including food manufacturing and genetic engineering. While grown in glucose-limited continuous cultures under aerobic growth conditions, researchers have observed autonomous oscillations in many extracellular and intracellular variables such as dissolved oxygen, glucose and ethanol concentrations, carbon dioxide evolution rate, oxygen uptake rate, cell size distribution, protein content and storage carbohydrates [4, 12, 56, 62, 64, 68, 80]. These oscillations are normally sustained: they can be maintained for many generations and the disappearance is usually attributable to external disturbances [61, 78]. Three types of autonomous oscillations have been reported in literature [56, 62, 64]: cell cycle dependent oscillations, glycolytic oscillations, and short-period-sustained oscillations. Only the cell cycle dependent oscillations are addressed in this work. These oscillations are also known to be strongly associated with cell cycle synchronization of the yeast population [68, 78]. Oscillations are reported to appear at intermediate dissolved oxygen levels [56, 68] and for a range of dilution rates, typically 0.09-0.25 hr⁻¹ [64]. The periods of oscillation varies from 2 to 45 hr depending on the particular strain and operating conditions [61, 78]. Existence of multiple stable stationary solutions under same operating conditions has also been reported [68, 81].

The underlying mechanisms that cause the oscillatory yeast dynamics are controversial and have been a subject of intensive research. Major research efforts has
been focused on the characterization of intracellular metabolisms, extra-cellular men-
dia, cell cycle and the interaction of cells with the environment. Depending on the
emphasis of study on these various aspects, a variety of dynamic models have been
proposed [16, 42, 56, 61, 77, 80]. However, no single reported model has been consid-
ered satisfactory in addressing all of the complex dynamics that are experimentally
observed.

Physically, a yeast culture is comprised of a population of individual cells with
different physiological and biochemical properties and an extracellular media with a
number of different components. In addition, a living cell is a very complex biochem-
ical system. Its growth and division involves a series of complicated chemical and
biochemical reactions such as uptake of substrate, exchange of oxygen and carbon
dioxide with the environment, accumulation of cell mass, doubling of the nuclei acid,
nuclear division and cell separation. Fredrickson [22] introduced the term “segre-
gated”, to indicate explicit accounting for the presence of heterogeneous individuals
in a cell population, and the term “structured” to designate the accounting for the
various intracellular and extracellular chemical components. As unsegregated and
unstructured models are over-simplistic, the large amount of existing models can be
classified into three categories: structured and unsegregated [14, 31, 42]; unstruc-
tured and segregated [11, 21, 34, 36]; and structured and segregated [15, 76, 77]. In
the following discussions, a few key features of these three classes of models will be
reviewed along with several representative models proposed in the literature.
Structured and unsegregated models consider the biophase as a continuous and well-mixed phase and account for for the chemical structure of the biophase. Jones and Kompala [42] have proposed a structured, unsegregated model to describe the growth dynamics of *S. cerevisiae* in both batch and continuous cultures. The model is derived using the cybernetic modeling framework first proposed by Ramkrishna and co-workers [69]. It is based on an underlying assumption that the biophase can always utilize the substrate in an optimal way to maximize the cell mass output. This framework offers a key advantage of being able to replace the detailed modeling of regulatory processes with the so-called cybernetic variables which represent the optimal strategies for enzyme synthesis and activity. Therefore it can significantly reduce the model complexity. In the cybernetic yeast model, the biophase utilizes the two substrates available in the media in three possible metabolic pathways: fermentative consumption of glucose, oxidative consumption of glucose and oxidative consumption of ethanol. All three metabolic pathways have been assumed available for the cell mass growth; however, the metabolic pathway with the highest growth rate dominates the growth. This model is able to produce sustained oscillation over a wide range of operating conditions. It is also able to predict an important experimentally observed feature that multiple stable solutions exist under certain operating conditions.

The cybernetic model has a few major drawbacks. First of all, as the model does no account for the segregated nature of the cell culture, it can not explain
the observed cell cycle synchrony which is believed to play a critical role in the stabilization of the oscillations [62, 68, 70]. Instead, it views the oscillations as a mere result of competitions among metabolic pathways. This explanation is questionable because it is based on the unexplained coincidences that the period of the oscillations match the characteristic time of the cell cycle and that the metabolic oscillations have magnitudes that will result in induction synchrony. The cybernetic model also predicts quick elimination and regeneration of metabolic oscillations in response to the changes in operating conditions. This was considered a strong evidence that the dynamic competition between the three metabolic pathways is indeed the causative factor for the oscillations. However, these predictions are not in accordance to experimental results.

In contrast, structured and segregated models attempt to explore the very details of the cell culture. Cells are differentiated individually and oscillations are viewed as a result of cell population dynamics rather than cell metabolism. The cell division of *S. cerevisiae* is asymmetric: the division produces two cells of unequal size, the smaller one is called a daughter cell and the larger one is called a mother cell. A representative model is proposed by Strässle et al. [77]. In this model the population was discretized by cell mass into a number of classes. Mother and daughter cells were identified by comparing cell mass to the cell mass required to form a bud. The concept of different metabolic pathways was also utilized. The model is based on a number of assumptions and experimental observations such as the respiratory
bottleneck hypothesis [77], accumulation of internal storage carbohydrates during the single cell phase and mobilization of these reserves during the budding phase, constant duration of the budding phase and asymmetric division. All these detailed considerations make the model a more realistic description of the physical system. The model is comprised of a set of cell mass distribution balances combined with a metabolic model that accounts for the basic variables. It is capable of predicting sustained oscillations with periods comparable to those observed experimentally. However, the model solution is quite laborious due to the requirement of a large number of discrete cell classes to obtain solutions in reasonable agreement with the experimental results. Some model parameters do not have clear physical meaning and can not be determined from experimental measurements. The complexity also make the model not well suited for practical control applications.

Unstructured and segregated models can be viewed as simplifications of the structured and segregated models by ignoring either the intracellular or extracellular chemical structures. Individual cells can be identified by a single variable such as cell age or cell mass. The cell growth kinetic is simply accumulation of cell mass with the consumption of substrates. The cell cycle can be simplified to be controlled by two discrete points denoting the start of budding and division. The key feature of these models is a population balance equation (PBE) that describes the time evolution of the cell age or cell mass distribution, combined with mass balance equations of other variables. Fredrickson et al. proposed the use of cell mass as advantageous over cell
age as the cell index [22]. A series of modeling work has been performed in the LSU Chemical Engineering department [35, 59, 81, 89] following the unstructured and segregated framework. All these models are able to predict sustained oscillations with cell cycle period values closely matching experimental results. The latest result [59] is also able to predict the coexistence of multiple stable solutions over a non-trivial range of operating conditions and is thus viewed as a major improvement over the others. More detailed descriptions of these models will be presented in later chapters.

1.3 Nonlinear Systems Dynamics and Bifurcation Analysis

A continuous-time dynamic system

\[
\frac{dx}{dt} \equiv \dot{x} = L(x), \quad x \in \mathbb{R}^n
\]  

(1.1)

is called a linear system if it satisfies the superposition principle

\[
L(c_1x_1 + c_2x_2) = c_1L(x_1) + c_2L(x_2), \quad \forall x_i \in \mathbb{R}^n, c_i \in \mathbb{R}, i = 1, 2
\]

Linear systems are more commonly expressed as \( \dot{x} = Ax \) where \( A \) is an \( n \times n \) matrix to indicate the linearity. Straightforwardly, any system

\[
\dot{x} = f(x)
\]  

(1.2)
that does not satisfy the superposition principle is called a nonlinear system. Rigorously, there is no linear systems existing in the real world. However, many physical systems can be well approximated by linear systems under certain conditions.

A linear system has many nice features such as the stability of its equilibria is global. The linearity greatly simplifies dynamic analysis and synthesis studies. The linear system theories have been well established and many powerful analysis tools have been developed. Therefore, whenever possible, it is always desirable to develop a linear model for a physical system. And the first step in analyzing an existing nonlinear system is usually to linearize it around some nominal operating point and analyze the resulting linear model. This is a useful practice and is commonly employed in engineering. There is no doubt that we should make use of linearization to learn as much as possible about the dynamic behaviors of a nonlinear system. However, there are two basic limitation of linearization that make it not sufficient to fully analyze a nonlinear system. First, linearization is a “local” approximation of the nonlinear system in the neighborhood of an operating point, it can only predict the local behavior of the nonlinear system in the vicinity of that point. Second, there are many “essentially nonlinear phenomena” that can take place only in a nonlinear system. Therefore, they can not be described or predicted by any linear model [43]. Some examples of nonlinear phenomena are:

- Multiple isolated equilibria: A linear system can have only one isolated equilibrium point and its stability is global. Therefore a linear system can have
only one steady-state solution that attracts the state of the system irrespective of the initial state. A nonlinear system can have more than one isolated zeros. The system state can converge to one of several stable attractors depending on the initial condition.

- Limit cycles: The only situation that a linear system can oscillate is that it has a pair of eigenvalues on the imaginary axis, which is a non-robust condition which is impossible to maintain in the presence of perturbations. And the oscillation amplitude is dependent on the initial condition. Stable oscillation with fixed period and initial condition independent amplitudes can only occur in nonlinear systems. This kind of oscillation is termed as a limit cycle.

- Multiple modes of behavior: It is not unusual for two or more modes of behavior to be exhibited by the a nonlinear system operating under same conditions. For example, a system may have more than one stable equilibrium and a stable limit cycle or multiple limit cycles. Hysteresis behavior can be observed if small perturbations are introduced to a system at an initial state near the separatrix of two stable attractors.

- Finite escape time: The state of an unstable linear system goes to infinity as time approaches infinity; a nonlinear system’s state can go to infinity in finite time.
• Chaos: A nonlinear system can have more complicated steady-state behavior that is not equilibrium or periodic oscillation. Such deterministic non-periodic behavior are referred to as chaos.

The first three of these phenomena will be encountered in the later study of yeast culture dynamic behaviors and will be discussed in more details.

As we know, systems of physical interest typically have parameters appearing in the system equations. A more general expression of a nonlinear system taking explicit account of operating parameters can be formulated as

\[ \dot{x} = f(x, \alpha), \quad x \in \mathbb{R}^n, \alpha \in \mathbb{R}^m \]  

(1.3)

It is often of practical interest to study the solution structure of a dynamic system under variation of these parameters. These solution structure includes (but is not limited to) properties such as the number of equilibria, stability of the equilibria, existence of limit cycles, multiple modes of behavior and chaos [46]. A significant difference between linear and nonlinear systems is that the solution structure of a nonlinear system can change under small perturbations of the model parameters at certain parameter values. In other words, a nonlinear system might be structurally unstable. These changes are called bifurcations and the parameter value at which the system is structurally unstable is called a bifurcation value or a bifurcation point. Commonly seen bifurcation examples include: a simple equilibrium, or fixed point attractor giving way to a periodic oscillation and a periodic attractor might
become unstable and be replaced by a chaotic attractor. More rigorous and complete descriptions of these concepts associated with bifurcation can be found in a number of excellent textbooks such as Kuznetsov [46] and Guckenheimer and Holmes [30]. A brief introduction will also be presented in Chapter 2.

(a) Fold bifurcation  
(b) Hopf bifurcation

Figure 1.1: Simplest bifurcations

Bifurcation analysis is simply the study of how the solution structure of a nonlinear dynamic system changes as key parameters are varied. The starting point of bifurcation analysis is typically to investigate the stability of equilibria and limit cycles which the most common bifurcations are associated with. Consider a continuous-time nonlinear system in Eq. (1.3) where $f$ is smooth with respect to both the state vector $x$ and the bifurcation parameter vector $\alpha$. If $x_0$ is a hyperbolic equilibrium point where all the real parts of the eigenvalues of the Jacobian matrix $Df(x_0)$ are non-zero, then a small perturbation in the model parameters will not change
the qualitative behavior of the system; i.e., a hyperbolic equilibrium is structurally stable. Bifurcations occur when some of the eigenvalues approach the imaginary axis in the complex plane. The simplest bifurcations are associated with a single real eigenvalue becoming zero ($\lambda_1 = 0$), referred to as a \textit{fold bifurcation} or a pair of complex conjugate eigenvalues crossing the imaginary axis ($\lambda_{1,2} = \pm i\omega_0, \omega_0 > 0$), referred to as \textit{Hopf bifurcation}. These are the most common bifurcations present in nonlinear systems. Fold bifurcations usually are the cause of multiple steady states and hysteresis behavior. Hopf bifurcations are responsible for the appearance and disappearance of periodic solutions. An illustrative example is shown in Figure 1.1, where $\mu$ stands for the bifurcation parameter, the $x$ branch in the first subplot stands for the trajectory of equilibrium solutions and the $a$ branch denotes the amplitude trajectory of periodic solutions in the second subplot.
Similar bifurcations exist for limit cycles as well. However, the study of limit cycles is more complicated. The concept of Poincaré map is introduced to facilitate this task. A Poincaré map effectively transforms a limit cycle of an \( n \)-dimensional continuous-time system to a fixed point of an \( (n-1) \)-dimensional discrete-time system by integrating the system states over a period of time, as shown in Figure 1.2. Stability of the limit cycle is then determined by examining the magnitude of the Floquet multipliers which characterize the stability of the linearized Poincaré map. There always is a single Floquet multiplier of unity magnitude due to the translational invariance of the solution in time. The periodic solution is stable if the remaining \( n-1 \) Floquet multipliers are inside the unit circle. Bifurcations of limit cycles occur when one or more Floquet multipliers cross the unit circle. More information can be found in [46].

While bifurcation theory is a powerful tool for model characterization, analytical treatment of physically-based models usually is intractable due to the complexity of the nonlinear model equations. A number of software packages including AUTO [20], LOCBIF [44] and CONTENT [47] have been developed for numerical bifurcation analysis of low-order nonlinear models. Beyond the simple bifurcations mentioned above, these packages are capable of locating more complex bifurcations and bifurcations associated with more than one bifurcation parameter. Numerical bifurcation analysis involves an iterative procedure known as continuation. First a stable steady-state or periodic solution for a particular set of parameter values is located
by dynamic simulation. Then one of the parameters is varied to allow the continued calculation of solutions as a function of this bifurcation parameter. At each iteration, a step in the bifurcation parameter is taken and a predictor-corrector method is utilized to locate the new solution. The step size of each iteration is controlled by a convergence criteria. The procedure is repeated until a desired range of parameter values has been evaluated. Therefore, continuation can provide a “complete” picture of the nonlinear dynamic behavior.

The results of the continuation calculations typically are presented as a bifurcation diagram where the solution structure of a key model variable is shown as a function of the bifurcation parameter. Since a bifurcation diagram provides a very concise and complete description of the system behavior, it is ideal for comparing model predictions to experimentally observed behavior. As several case studies in Chapter 2 will illustrate, bifurcation analysis is much more effective and efficient for nonlinear systems dynamic analysis than the traditional way of using open loop simulations. Although the task of full investigation of many complex systems is rarely possible, partial knowledge of the bifurcation diagram still can provide very important information on the behavior of the system being studied.

1.4 Nonlinear Model Order Reduction Techniques

Dynamic models of high dimension are not rare in study of chemical systems. When a first principle model consists of partial differential equations (PDE), it is essentially
infinite-dimensional. Accurate discretization of these models will usually result in models of very high dimension. The number of ODEs is often in the order of $10^{2n}$, where $n$ stands for the number of independent variables in the PDE model [9]. The model size not only dramatically increases computational cost but also frequently make the model ill-conditioned and introduce numerical errors during integration. Even though current computer technology allow for the routine simulation of such systems, nonlinear dynamic analysis and controller design based on such full scale models are practically impossible. Fortunately, the long-term dynamics of most high dimensional systems are effectively low-dimensional. Investigation of the spectrum of the linearized operator of such a nonlinear system will indicate a clear separation of eigenvalues in the complex space [43]. The many “fast modes” corresponding to those eigenvalues far away from the imaginary axis in the right half plane will relax very rapidly and the relevant system dynamic behavior will be dominated by the “slow modes” associated with the other eigenvalues. Therefore, the long term dynamics of the full scale model could be represented by a small set of ODEs. Geometrically, the transient system trajectory will converge very quickly to a manifold of the same dimension as the number of slow modes. The practice of nonlinear model order reduction is basically to exploit the time-scale separation of the system modes and to approximate the long-term behavior by a system of much smaller dimension consisting of the slow modes only. The accuracy of the reduced-order model (ROM) can be checked in various ways.
Many nonlinear model reduction techniques have been proposed in the literature. Most of these methods can be categorized into three groups: (i) methods based on proper orthogonal decomposition (POD) [37]; (ii) methods based on approximate inertial manifolds (AIM) [7] and (iii) methods based on balanced truncation [48].

The POD method was originally proposed by Pearson [67] and has been popularized by Lumley and others [37] for the study of dynamical features in complex fluid flows. More recently, POD has found to be very successful in solids and structures [37], image processing [74] and the design of controllers for PDE systems [73]. It is also known as empirical orthogonal eigenfunctions (EOF), Karhunen-Loève expansion (KLE), principal component analysis (PCA), factor analysis, and total-least-squares estimation in the literature. The basic idea underneath the POD method is to extract a set of global basis functions (i.e., the EOFs) through statistical analysis (essentially PCA) of extensive empirical data (simulation or actual process data). The basis function set is then used in a projection procedure (usually the Galerkin weighted residual projection) of the full model. The PCA approach ensures that the POD method is optimal in the sense that the empirical eigenfunction set will capture as much of the system "energy" as possible given the number of basis functions retained in the subspace. Clearly there is a trade-off between the extent of model reduction and the quality of the reduced-order model which have to be determined case by case.
Since the POD method is totally based on empirical data, the collection of a representative data set is then crucial for the success of applying this method. The data ensemble is the starting point for forming the reduced-dimensional subspace onto which the original state space is projected by the Galerkin procedure. All dynamics orthogonal to this subspace are neglected under the assumption that the resulting error will be small. In addition to the necessity for a large spatiotemporal data set, there are no a priori comprehensive guidelines for generation of a suitable ensemble from which the empirical basis functions will be extracted. A potentially representative ensemble can be obtained by combining spatiotemporal motions at several values of key operating parameters [19], mixing transients from different initial conditions distributed randomly around relevant regions of the phase space [28] and collecting responses to perturbation of actuators from their nominal settings [6]. Reduced-order models obtained from such data set is expected not only to be capable of capturing the short-term and long-time dynamics of the systems, but also to account for the effect of model parameters and inputs on the system states indirectly [73].

An inertial manifold is defined as a smooth finite-dimensional invariant manifold which exponentially attracts all orbits in the infinite-dimensional phase space and thus contains the global attractor [40, 43]. The existence of inertial manifolds has been established for many dissipative PDE system. However, there is no theory to provide explicit solutions of the inertial manifolds. In order to implement it compu-
tationally, an approximation is necessary. There are two categories of approximation schemes in the literature: those approximate a true inertial manifold [23, 55] and those approximating the global attractor [24, 40]. The manifold in either case are referred to as approximate inertial manifold (AIM). In the latter approach, AIMs can be used even if an inertial manifold does not exist. Another advantage of the AIM method is that the eigenfunctions can generally be derived analytically from the linear differential operator of the PDE system, thus avoiding the need of accurate discretization as in the POD method. The eigenfunctions are often in the form similar to a Fourier series. The AIM is then obtained by truncating the eigenfunction set using certain projection methods. One disadvantage of this method is that the system might have multiple inertial manifolds and the eigenfunction set might not be optimal. Many researchers have confirmed that an AIM based model usually requires significantly more basis functions than POD base model [7, 73].

A severe limitation of AIM and POD based methods is that the input-output behavior of the system is not taken into account, which makes the reduced-order model less attractive for control purpose. This limitation intrigues the study of the balance truncation method.

The method of balance truncation for model reduction of linear systems was proposed by Moore [60] and is now a well-developed and standard method of model reduction [87]. For linear systems, this method only requires matrix computation and has been successfully used in control design. The use of balance truncation
method for nonlinear system has attracted more attention since Sherpen [71, 72].
For nonlinear systems, most methods in the literature requires the computation of energy functions which is difficult under most situations [63]. Lall et al. [48] proposed a data-based approach which combines the features of POD method and balance truncation. The essence of their method is to compute the empirical controllability gramian and empirical observability gramian using data from simulation or experiment, thereby to construct an approximate balanced realization. This approach avoids the computation of energy functions and requires only standard matrix operations.

The key advantage of balance truncation methods over POD and AIM based method is that it takes explicit account of the impact from inputs on the system dynamic behaviors. Therefore the resulting reduced-order model is able to preserve the input-output relationship of the original system. However, the balanced truncation method is not optimal. Further more, for high order systems, the ill-conditioning of the full model often results in rank-deficient empirical gramians which makes the balanced truncation method unimplementable.
Chapter 2

Bifurcation Analysis of Continuous Bioreactor Models

2.1 Introduction

Biochemical reactors can be viewed as highly complex dynamic systems. The intracellular and extracellular environments are comprised of many chemical components and each cell has unique properties. A rigorous mechanistic model accounting for both these complexities is known as a structured and segregated model [15, 22, 76]. Such models consist of coupled sets of partial differential equations and ordinary differential equations. In addition to being difficult to formulate, these models are not amenable to systematic analysis due to their complexity. Simplified models can be developed either by neglecting the intracellular chemical structure (unstructured segregated models) [11, 21, 35] or by neglecting heterogeneity of the cell population (structured unsegregated models) [14, 42, 69]. The simplest models are both unstructured and unsegregated [18]. If the intracellular environment can be characterized by a few critical variables [42, 69], then structured unsegregated models (like unstructured unsegregated models) consist of a reasonably small number of nonlinear ordinary differential equations. Such models are well suited for rigorous analysis.
Three unsegregated models of different cell cultures are studied in this chapter. First the structured model of Hybridoma cells proposed by Guardia and co-workers [29] is considered. This mammalian cell culture is reported to exhibit multiple steady states under certain operating conditions [26, 88]. The cybernetic modeling paradigm used to reproduce this behavior involves the maximization of cell growth via competition between alternative pathways which utilize a pair of complementary and partially substitutable substrates. An unstructured model proposed by McLellan and co-workers [57] for continuous fermentation of the microorganism Zymomonas mobilis is studied next. Continuous cultures of this microorganism exhibit oscillatory behavior which adversely affect ethanol productivity and reactor operability. This behavior is captured in the model by introducing a dynamic specific growth rate which accounts for the inhibitory effect of the past ethanol rate of change on the current state of the reactor. The last model investigated is a structured model proposed by Jones and Kompala [42] for the yeast Saccharomyces cerevisiae. Continuous cultures of this microorganism have been shown to exhibit complex dynamic behavior that includes the sudden appearance and disappearance of sustained oscillations [64, 78, 80]. The cybernetic model reproduces this behavior through the competition of three metabolic pathways that are utilized to maximize the cell growth rate.

Transient bioreactor models often are evaluated by comparing experimental data and model simulation results. While dynamic simulation is a very useful tool for
model validation, several limitations can be identified: (i) it is inefficient and potentially inconclusive, especially when the model possesses slow dynamic modes; (ii) it is necessarily incomplete since only a limited number of simulation tests can be performed and important dynamic behaviors may not observed; and (iii) it does not easily reveal the model characteristics that lead to certain dynamic behaviors. Therefore, dynamic simulation should not be viewed as the only tool for evaluating transient bioreactor models.

The purpose of this chapter is to demonstrate that bifurcation analysis is a powerful tool for evaluating transient models of continuous bioreactor. The objective of bifurcation theory is to characterize changes in the qualitative dynamic behavior of a nonlinear system as key parameters are varied. The model equations are used to locate steady-state solutions, periodic solutions and bifurcation points where the qualitative dynamic behavior changes. Bifurcation analysis can be much more effective than simply integrating the model equations over time and comparing the transient responses to experimental data. Instead a “complete” picture of the model behavior is obtained in the form of a bifurcation diagram. This diagram can be used to determine if the model supports the steady-state and dynamic behavior observed experimentally. It also can guide the design of experiments aimed at validating unexpected model predictions. Numerical bifurcation packages developed by a number of researchers [20, 44, 47] make bifurcation analysis of low-order nonlinear systems reasonably simple.
It is important to note that a number of other investigators have applied bifurcation analysis to continuous bioreactor models [1, 53, 65, 86] of these studies focus on the dynamics of two microbial populations competing for a common rate limiting substrate [2, 3, 49, 66]. In most studies the objective is to provide a very detailed characterization of the model behavior with minimal comparison to available experimental data. The objective of study in this chapter is notably different. This study is concerned primarily with the use of bifurcation analysis for validation of transient bioreactor models. The comparison of physically meaningful model behavior with experimental data is emphasized rather than cataloging all possible model behaviors. A similar approach has been pursued by Jones and Kompala [42] to determine unknown parameters in their cybernetic yeast model. The present contribution can be viewed as an extension of the bifurcation studies on unstructured cell population balance models for continuous yeast bioreactors [85].

The remainder of this chapter is organized as follows. Basic concepts of bifurcation theory and an introduction to the bifurcation package AUTO are presented in Section 2.2. The dynamic behavior and bifurcation analysis of the aforementioned bioreactor models are discussed in Sections 2.3–2.5. A summary and conclusions are presented in Section 2.6.

2.2 Bifurcation Analysis

A nonlinear dynamic system differs from a linear dynamic system in that its qualitative properties can change under small perturbations of the model parameters,
These properties include the number of equilibria, stability of the equilibria, existence of limit cycles, multiple modes of behavior and chaos [46]. Below is a very brief introduction to bifurcation analysis. The textbook by Kuznetsov [46] provides more complete descriptions of these concepts. A bifurcation is introduced formally as follows.

**Definition 1.** Two dynamical system $F : \mathcal{R}^n \rightarrow \mathcal{R}^l$ and $G : \mathcal{R}^n \rightarrow \mathcal{R}^l$ are called topologically equivalent if there exists a diffeomorphism $h : \mathcal{R}^n \rightarrow \mathcal{R}^n$ such that $h \circ F = G \circ h$.

Two topologically equivalent systems have the same qualitative dynamic behavior in the sense that they can be mapped to each other.

**Definition 2.** The appearance of a topologically non-equivalent phase portrait under variation of a parameter is called a *bifurcation*.

Bifurcation analysis is the study of how the qualitative properties of a nonlinear dynamic system change as key parameters are varied. Consider a continuous-time nonlinear system depending on a parameter vector $\alpha$:

$$\dot{x} = f(x, \alpha), \quad x \in \mathcal{R}^n, \alpha \in \mathcal{R}^l \quad (2.1)$$

where $f$ is smooth with respect to both the state vector $x$ and the bifurcation parameter vector $\alpha$. If $x_0$ is a hyperbolic equilibrium point where all the real parts of the eigenvalues of the Jacobian matrix $Df(x_0)$ are non-zero, then a small perturbation in the model parameters will not change the qualitative behavior of the system; *i.e.*
a hyperbolic equilibrium is structurally stable. Bifurcations occur when some of the
eigenvalues approach the imaginary axis in the complex plane. The simplest bifur-
cations are associated with a single real eigenvalue becoming zero ($\lambda_1 = 0$) or a pair
of complex conjugate eigenvalues crossing the imaginary axis ($\lambda_{1,2} = \pm i\omega_0, \omega_0 > 0$).

**Definition 3.** The bifurcation where $\lambda_1 = 0$ is called a *fold* bifurcation.

**Definition 4.** The bifurcation where $\lambda_{1,2} = \pm i\omega_0, \omega_0 > 0$ is called a *Hopf* bifurca-
tion.

These are the most common bifurcations present in nonlinear systems. Fold bi-
furcations usually are the cause of multiple steady states and hysteresis behavior.
Hopf bifurcations are responsible for the appearance and disappearance of periodic
solutions.

While bifurcation theory is a powerful tool for model characterization, analytical
treatment of physically-based models usually is intractable due to the complexity of
the nonlinear model equations. A number of software packages including AUTO [20],
LOCBIF [44] and CONTENT [47] have been developed for numerical bifurcation
analysis of low-order nonlinear models. Numerical bifurcation analysis involves an
iterative procedure known as *continuation*. First a stable steady-state or periodic
solution for a particular set of parameter values is located by dynamic simulation.
Then one of the parameters is varied to allow the continued calculation of solutions as
a function of this bifurcation parameter. At each iteration, a step in the bifurcation
parameter is taken and a predictor-corrector method is utilized to locate the new
solution. The step size of each iteration is controlled by a convergence criteria. The procedure is repeated until a desired range of parameter values has been evaluated. Therefore, continuation can provide a “complete” picture of the nonlinear dynamic behavior.

The results of the continuation calculations typically are presented as a bifurcation diagram where the behavior of a key model variable is shown as a function of the bifurcation parameter. The steady-state and periodic solutions are mapped into this two-dimensional space. As compared to dynamic simulation, a key advantage of continuation is that unstable as well as stable solutions can be located. Because it provides a very concise and complete description of the system behavior, a bifurcation diagram is ideal for comparing model predictions to experimentally observed behavior. For example, the range of model parameter values that support multiple steady states or periodic solutions can be determined. This allows a more meaningful analysis of a nonlinear model than is possible with dynamic simulation alone. In the next three sections, the applicability of bifurcation analysis to continuous bioreactor models is demonstrated via three example systems.

The AUTO continuation package developed by Doedel and co-workers [20] is perhaps the most widely used numerical bifurcation code. AUTO can perform bifurcation analysis of nonlinear systems described by algebraic equations, ordinary differential equations and parabolic partial differential equations. In addition to the simple fold and Hopf bifurcations described above, AUTO can locate tori and
period doubling bifurcations as a function of two or more parameters. AUTO also includes a graphical user interface (GUI) which simplifies specification of computational parameters required by the continuation code. For these reasons, AUTO is used in this chapter. It is important to note that AUTO and other general purpose bifurcation codes can be expected to work only for nonlinear models of moderate dimension. Therefore, they are best suited for analysis of unstructured, unsegregated and simple structured, unsegregated bioreactor models.

2.3 Hybridoma Cell Bioreactor Model

2.3.1 Background

Hybridoma cells utilize glucose and glutamine as complementary and partially substitutable substrates for growth [8, 82]. There exists several metabolic pathways, each of which is favored under certain culture conditions, for cell growth. The existence of these multiple pathways creates complex behavior when Hybridoma cells are grown in a continuous bioreactor. In particular, researchers have shown that different steady states can be reached when cultures with the same operating conditions but different initial metabolic states are switched from batch or fed-batch mode to continuous operation [26, 88].

A structured, unsegregated model has been proposed by Guardia et al. [29] to capture the observed steady-state multiplicity. The cybernetic model accounts for the multiple metabolic pathways created by the complementary and partially
substitutable substrate utilization. The cybernetic model predicts the existence of multiple steady-state solutions for some operating conditions. More specifically, it is shown that different initial conditions established by batch and fed-batch operation can lead to different steady-state solutions. However, the authors note that this property is very sensitive to changes in the model parameters and the region of operating conditions that support multiplicity is quite small. It is not clear if this lack of robustness is attributable to the model structure or to the particular choice of model parameters. Furthermore, a precise characterization of the operating range which supports steady-state multiplicity has not been presented.

2.3.2 Transient Model

Ramkrishna and co-workers [69] have proposed that microorganisms optimize utilization of available substrates to maximize their instantaneous growth rate. While somewhat controversial, this hypothesis has been derived from the analysis of extensive experimental data. The cybernetic modeling approach has been used to capture the partially substitutable and complementary substrate utilization that leads to multiple metabolic pathways in Hybridoma cultures. A detailed description of the metabolic pathways can be found in the original reference [29]. The cybernetic model includes: two substrates, glucose and glutamine; three intermediates; and five enzymes associated with the various pathways.

The cybernetic model equations are:
\[
\frac{dX}{dt} = (r_g - D)X \\
\frac{dS_1}{dt} = -(r_1v_1^c + r_3v_3^c)X + D(S_1^f - S_1) \\
\frac{dS_2}{dt} = -r_2v_2^gX + D(S_2^f - S_2) \\
\frac{dM_1}{dt} = Y_1r_1v_1^s + Y_4r_5v_5^s - (Y_{m1x} + M_1)r_g - r_4v_4^s \\
\frac{dM_2}{dt} = Y_2r_2v_2^s + Y_5r_4v_4^s - (Y_{m2x} + M_2)r_g - r_5v_5^s \\
\frac{dM_3}{dt} = Y_3r_3v_3^s - (Y_{m3x} + M_3)r_g \\
\frac{de_i}{dt} = r_{ei}^* + r_{ei}u_i^e u_i^a - (r_g + b_i)e_i \quad i = 1, \ldots, 5
\]  

(2.2)

where: \( X \) is the cell mass concentration; \( S_1 \) and \( S_2 \) are the concentrations of glucose and glutamine, respectively; \( M_i \) and \( e_i \) are the concentrations of the three intermediates and five enzymes, respectively; \( S_1^f \) are the feed substrate concentrations; \( D \) is the dilution rate; \( Y_i \) are the yield coefficients; \( r_{ei}^* \) is the constitutive synthesis rate of the enzyme \( e_i \); and \( b_i \) is the degradation rate constant of the enzyme \( e_i \). The cybernetic variables \( u_i^e, u_i^s, v_i^e \) and \( v_i^s \) are the synthesis and activity coefficients of the enzymes for the complementary and substitutable pathways, respectively. The reaction rates \( r_i \) and \( r_{ei} \) are assumed to follow Monod-type kinetics. Their definitions can be found in the original reference [29] and are omitted here for sake of brevity.
Difficulties were experienced in producing multiple steady-state solutions with the model parameter values reported in [29]. After modifying several parameter values, the expected multiple steady-state behavior were generated. The parameter values used in this simulation and bifurcation studies are listed in Table 2.1 where the values of $r_i^{\text{max}}$ and $Y_{M,x}$ are different from those listed in the original reference. The discrepancy between the two sets of parameter values further motivates the need for a more detailed investigation of the model behavior using bifurcation analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_i^{\text{max}}$</td>
<td>g/gdw-h</td>
<td>0.05, 0.03, 0.01, 0.03, 0.01</td>
</tr>
<tr>
<td>$K_i$</td>
<td>g/L</td>
<td>0.001, 0.001, 0.001, 0.01, 0.0001</td>
</tr>
<tr>
<td>$r_{ei}^{\text{max}}$</td>
<td>g/gdw-h</td>
<td>0.001, 0.0005, 0.001, 0.0005, 0.001</td>
</tr>
<tr>
<td>$K_{ei}$</td>
<td>g/L</td>
<td>0.001, 0.0001, 0.001, 1e-5, 1e-5</td>
</tr>
<tr>
<td>$r_{ei}^*$</td>
<td>g/gdw-h</td>
<td>1e-6, 1e-6, 1e-6, 1e-6, 1e-6</td>
</tr>
<tr>
<td>$b_i$</td>
<td>gdw/gdw-h</td>
<td>0.05, 0.1, 0.1, 0.1, 0.05</td>
</tr>
<tr>
<td>$Y_i$</td>
<td>g/g</td>
<td>0.9, 0.9, 0.8, 1.0, 1.0</td>
</tr>
<tr>
<td>$K_{gM}$</td>
<td>g/gdw</td>
<td>0.0005, 0.0005, 0.0005</td>
</tr>
<tr>
<td>$Y_{M,x}$</td>
<td>g/gdw</td>
<td>0.7, 0.99, 0.1</td>
</tr>
<tr>
<td>$r_q^{\text{max}}$</td>
<td>gdw/gdw-h</td>
<td>0.0575</td>
</tr>
</tbody>
</table>

### 2.3.3 Results and Discussion

Several dynamic simulation tests were performed to study the steady-state behavior of the cybernetic model in different regions of the operating space. In the first test, the reactor initially is at a steady state corresponding to $S_1^f = 0.95$ g/L, $S_2^f = 0.43$ g/L, and $D = 0.0295$ h$^{-1}$. The results obtained for step changes of various magnitudes in the dilution rate are shown in Figure 2.1 where the cell mass
concentration \((X)\) is selected as a representative output variable for the culture. The steady states obtained for \(D = 0.0300 \text{ h}^{-1}\) and \(D = 0.0292 \text{ h}^{-1}\) are close to the initial steady state, while those for \(D = 0.0291 \text{ h}^{-1}\) and \(D = 0.0290 \text{ h}^{-1}\) are very far removed. Also shown is the response obtained for a step change to \(D = 0.0300 \text{ h}^{-1}\) at \(t = 2000 \text{ h}\) from the \(D = 0.0290 \text{ h}^{-1}\) steady state. The culture reaches a different steady state than the one obtained for the \(D = 0.0300 \text{ h}^{-1}\) step change from the initial steady state.

These dynamic simulation results demonstrate that the cybernetic model predicts the presence of multiple stable steady states and hysteresis behavior. On the
Figure 2.2: Bifurcation diagram of the Hybridoma reactor model.

On the other hand, this type of analysis provides a rather incomplete picture of the model behavior. For instance, dynamic simulation is not a convenient tool for determining the range of dilution rates over which multiple steady states exist. This is very valuable information for model validation. It is shown below that bifurcation analysis is a powerful tool for extracting this knowledge.

Figure 2.2 shows the one-parameter bifurcation diagram for the cybernetic model where the dilution rate \( D \) is chosen as the bifurcation parameter and the cell mass concentration \( X \) is the output variable. The feed substrate concentrations are held constant at \( S_1^f = 0.95 \text{ g/L} \) and \( S_2^f = 0.43 \text{ g/L} \). The locus of steady-state operating points as a function of \( D \) is determined using AUTO. The solid line (—) denotes stable steady-state solutions, while the dashed line (−−) denotes unstable steady-
state solutions. The model exhibits fold bifurcations at $D = 0.02917 \text{ h}^{-1}$ (point 1) and $D = 0.03068 \text{ h}^{-1}$ (point 2) which delineate the parameter space where multiple steady-state solutions exists. In this region the model shows hysteresis behavior due to the S-shaped steady-state locus. There only is a single stable steady-state solution outside this region.

The bifurcation diagram clearly shows the existence of multiple steady-state solutions and provides an explanation for the hysteresis behavior observed in Figure 2.1. Each stable steady state has a domain of attraction from which all initial conditions converge to that steady state. Initial conditions corresponding to low dilution rates converge to the upper steady state while those corresponding to high dilution rates converge to the lower steady state. The bifurcation diagram also shows that the parameter region which supports multiple steady-state solutions is quite small: $D \in [0.02917 \text{ h}^{-1}, 0.03068 \text{ h}^{-1}]$. This is much more valuable information for model validation than simply knowing the model exhibits multiple steady states. In particular, experimental determination of the dilution rates that support multiple steady states can be used to adjust the model parameters to obtain agreement. If this is not possible, the model structure may be concluded to be inadequate. Such conclusions are very difficult to obtain from dynamic simulation alone.
2.4 *Zymomonas mobilis* Reactor Model

2.4.1 Background

*Zymomonas mobilis* has been proposed as a more promising microorganism than conventional baker’s yeast for industrial production of ethanol [10, 13]. A major drawback of this microorganism is that it exhibits sustained oscillations over a wide range of operating conditions when grown in continuous culture. This leads to decreased ethanol productivity and less efficient use of available substrate [13, 27]. Various models have been proposed to describe the oscillatory dynamics of continuous *Zymomonas mobilis* cultures [18, 27, 39, 50]. Daugulis *et al.* [18] present an unstructured, unsegregated model based on the concept of a “dynamic specific growth rate”. The predictive capability of the model has been evaluated experimentally by McLellan *et al.* [57]. A variety of simulation tests were performed and model predictions were found to be in reasonable agreement with experimental data. However, a more thorough analysis of the model dynamics with respect to the mathematical cause of the oscillations and the range over which periodic solutions exist has not been presented. It is shown below that bifurcation analysis is well suited to answer these important questions.

2.4.2 Transient Model

Daugulis *et al.* [18] argue that many models for continuous *Zymomonas mobilis* cultures require measurements of physiological quantities which are difficult and time
consuming to obtain. As an alternative, they propose the concept of a “dynamic specific growth rate” which explicitly accounts for the effect of past culture conditions on subsequent cell behavior. Based on this concept, a simple transient model that requires only measurements of extracellular variables such as ethanol, substrate and cell mass concentrations is proposed. The unstructured, unsegregated model consists of material balances on biomass, ethanol and substrate combined with two additional equations that describe the inhibition of cell growth caused by the past rate of change of the ethanol concentration. The model equations are:

\[
\begin{align*}
\frac{dX}{dt} &= [\mu(S, P, Z) - D]X \\
\frac{dS}{dt} &= -\left(\frac{1}{Y_{p/s}}\right) Q_p X + D(S_f - S) \\
\frac{dP}{dt} &= Q_p X - DP \\
\frac{dZ}{dt} &= \beta(W - Z) \\
\frac{dW}{dt} &= \beta(Q_p X - DP - W)
\end{align*}
\] (2.3)

where: \(X\), \(S\) and \(P\) are the biomass, substrate and ethanol concentrations, respectively; \(W\) and \(Z\) are the first-order and second-order weighted averages, respectively, of the ethanol concentration change rate; \(D\) is the dilution rate; \(S_f\) is the feed substrate concentration; \(\mu(S, P, Z)\) is the dynamic specific growth rate; \(Q_p\) is the specific production rate; \(Y_{p/s}\) is the yield coefficient between glucose and ethanol; and \(\beta\) is a parameter that determines the magnitude of the time lag for the delayed
inhibition effect. More precise definitions of $W$, $Z$, $\mu$ and $Q_P$ can be found in the original reference [57]. The model parameters used in the following simulation and bifurcation analysis are taken from the original reference and are listed in Table 2.2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{max}$</td>
<td>0.41 h$^{-1}$</td>
<td>$Q_{p,max}$</td>
<td>2.613 h$^{-1}$</td>
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<tr>
<td>$P_{ob}$</td>
<td>50.0 g/L</td>
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<td>$P_{ma}$</td>
<td>217.0 g/L</td>
<td>$K_{mp}$</td>
<td>0.5 g/L</td>
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<td>$P_{mb}$</td>
<td>108.0 g/L</td>
<td>$K_i$</td>
<td>200.0 g/L</td>
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<tr>
<td>$P_{me}$</td>
<td>127.0 g/L</td>
<td>$Y_{P/S}$</td>
<td>0.495 g/g</td>
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<tr>
<td>$S_i$</td>
<td>80.0 g/L</td>
<td>$\beta$</td>
<td>0.0366 h$^{-1}$</td>
</tr>
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<td>$\delta$</td>
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<td>$\lambda$</td>
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<td>$b$</td>
<td>1.415</td>
<td></td>
<td></td>
</tr>
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</table>

### 2.4.3 Results and Discussion

Dynamic simulations were performed to study the oscillatory behavior of the model in different regions of the operating space. Figure 2.3 shows two simulation tests at high dilution rates. The cell mass concentration ($X$) are chosen as the output variable for the culture. Both simulations start at the steady state corresponding to $D = 0.1120$ h$^{-1}$ and $S_f = 200$ g/L. The feed substrate concentration is maintained at this value and the dilution rate is changed to a slightly lower value. For a step change to $D = 0.1116$ h$^{-1}$, the culture oscillates with very small amplitude and the oscillations slowly decay to a steady-state solution which is close to the initial point. For $D = 0.1115$ h$^{-1}$, the culture exhibits sustained oscillations of a rather small
Figure 2.3: Dynamic simulation of the Zymomonas mobilis reactor model at high dilution rates.

amplitude. Note that the change in dynamic behavior for the two dilution rates is not dramatic. While these tests imply that the model exhibits a bifurcation in this region, it is a time consuming task to find by dynamic simulation the dilution rate at which this bifurcation occurs.

Figure 2.4 shows the results of three simulation tests at lower dilution rates and the same constant feed substrate concentration. The initial condition for the first two tests corresponds to the steady state for $D = 0.033$ h$^{-1}$. For a step change to $D = 0.0332$ h$^{-1}$, the culture oscillates with very small amplitude until a new
steady state is reached after a very long period of time. For $D = 0.0333 \text{ h}^{-1}$, the culture initially oscillates with small amplitude but eventually exhibits a transition to large amplitude oscillations. In the third test, the simulation is restarted from an oscillatory initial condition from the last test ($D = 0.0333 \text{ h}^{-1}$) and the dilution rate is stepped back to $D = 0.0332 \text{ h}^{-1}$. Sustained oscillations are maintained despite the fact that the same dilution rate produced a steady state in the first test.

These simulations verify that the model predicts the appearance and disappearance of sustained oscillation as observed experimentally. However, the results also
Figure 2.5: One-parameter bifurcation diagram of the Zymomonas mobilis reactor model.

raise questions about: (i) the large difference in amplitudes observed when sustained oscillations are initiated at low and high dilution rates; and (ii) the two different stable steady-state solutions present at the dilution rate $D = 0.0332 \text{ h}^{-1}$. Another issue that warrants further investigation is the use of multiple sets of parameter values for stationary and oscillatory simulations in the original reference [57]. It is shown below that bifurcation analysis provides a convenient framework to address these questions.

Figure 2.5 shows a one-parameter bifurcation diagram of the Zymomonas mobilis model where the feed substrate concentration ($S_f$) is held constant at 200 g/L. The dilution rate ($D$) is chosen as the bifurcation parameter, and the cell mass
concentration \((X)\) is selected as the output variable. At low dilution rates there only is a single stable steady-state solution \((-\)\). At point 2, a bifurcation occurs where the steady-state solution becomes unstable \((-\)\) and a periodic solution of the amplitude indicated is created. At point 1, another bifurcation occurs where the periodic solution disappears and the steady-state solution becomes stable. At higher dilution rates, there is a single stable steady-state solution.

The bifurcation that occurs at point 1 \((D = 0.11151 \text{ h}^{-1})\) is known as a supercritical Hopf bifurcation where loss of stability of the steady-state solution is accompanied by the appearance of very small amplitude oscillations. For the range of dilution rates between points 1 and 2, a stable periodic solution coexists with an unstable steady-state solution. The bifurcation that occurs at point 2 \((D = 0.03322 \text{ h}^{-1})\) is known as a subcritical Hopf bifurcation where large amplitude oscillations appear when the steady-state solution becomes unstable. Points 3 and 4 \((D = 0.03095 \text{ h}^{-1})\) represent a fold bifurcation of limit cycles. They denote the dilution rate at which the periodic solution changes stability. For dilution rates between points 2 and 3, stable and unstable periodic solutions coexist. Below point 3, the steady-state solution is the only stable solution of the model.

The one-parameter bifurcation diagram shows that the appearance and disappearance of periodic solutions is due to the existence of two Hopf bifurcation points. The analysis also answers the questions raised about the different amplitudes of sustained oscillations at low and high dilution rates. Since the Hopf bifurcation at
the lower dilution rate is subcritical, stable periodic solutions are characterized by large magnitude oscillations and the stable periodic solution coexists with a stable steady-state solution over a very small range of dilution rates. By contrast, the Hopf bifurcation at the higher dilution rates is supercritical and the associated periodic solutions have very small amplitude.

Further characterization of the model behavior can be obtained by computing the locus of each Hopf bifurcation point in the $D$ and $S_f$ plane. This is known as a two-parameter bifurcation diagram, and it allows the range of operating conditions under which periodic solutions exist to be determined. The two-parameter bifurcation diagram is shown in Figure 2.6. The upper branch between points 1 and 4 represents the locus of the supercritical bifurcation point, while the lower branch between points 5 and 6 is the locus of the subcritical bifurcation point. These two branches define a closed region in the $D$–$S_f$ plane in which stable periodic solutions exist and stable steady-state solutions cannot exist. Outside this region, sustained oscillations occur over a very small range of operating conditions and coexist with stable steady-state solutions. The diagram allows concise determination of the operating conditions which support sustained oscillations and is well suited for validating the model against data. It is very difficult to obtain this type of information using only dynamic simulation.

The two-parameter bifurcation analysis also provides insights into the structural limitations of the model. In the original reference [57], three sets of model parameters
Figure 2.6: Two-parameter bifurcation diagram of the *Zymomonas mobilis* reactor model.

were estimated to fit data when either the steady-state solution or the periodic solution was stable. The parameter values used in this study were obtained in [57] from oscillatory data. While each set of parameters provides a reasonable fit to the corresponding data, the lack of validation tests raises concerns about the simple model structure. In one set of experiments [57], the culture reaches a steady state for \( D = 0.133 \text{ h}^{-1} \) and \( S_f = 150 \text{ g/L} \). By contrast, the two-parameter bifurcation diagram in Figure 2.6 indicates that a periodic solution is expected for these values. This indicates that the model cannot be reconciled against experimental data and a more sophisticated model structure is needed. In fact, the authors [57] note that
oscillatory behavior is associated with a change in cell morphology. This suggests that a segregated model may be more appropriate.

2.5 *Saccharomyces cerevisiae* Reactor Model

2.5.1 Background

*Saccharomyces cerevisiae* (baker’s yeast) is an important microorganism in a number of industries including brewing, baking, food manufacturing and genetic engineering. Many investigators have shown that continuous cultures of *Saccharomyces cerevisiae* exhibit sustained oscillations in glucose limited environments under aerobic growth conditions [62, 64, 78, 80]. The underlying cellular mechanisms that cause oscillatory yeast dynamics are controversial and have been a subject of three decades of intensive research. A large number of transient models have been proposed to explain the existence of sustained oscillations [42, 78, 89]. Jones and Kompala [42] have proposed a cybernetic model which is able to predict the appearance and disappearance of sustained oscillations in continuous yeast bioreactors. A variety of simulation tests were performed to evaluate the dynamic behavior of the model. Such tests provide an incomplete characterization of the model properties which is biased towards the specific behaviors being investigated. It is shown below that a detailed bifurcation analysis can reveal complex dynamic behavior that is unlikely to be discovered using dynamic simulation alone.
2.5.2 Transient Model

Based on the cybernetic modeling framework [69], Jones and Kompala [42] have developed a structured, unsegregated model for continuous yeast bioreactors. Sustained oscillations are viewed as the result of competition between three metabolic pathways: glucose fermentation, ethanol oxidation and glucose oxidation. Detailed modeling of the intracellular regulatory processes is replaced by cybernetic variables $u_i$ and $v_i$ representing the optimal strategies for enzyme synthesis and activity, respectively. Denoting the instantaneous growth rate along the $i$-th pathways as $r_i$, the optimal strategies for $u_i$ and $v_i$ are:

$$u_i = \frac{r_i}{\sum_j r_j}$$
$$v_i = \frac{r_i}{\max_j r_j}$$

If the growth rate along the $i$-th pathway is large, then the associated synthesis ($u_i$) and activity ($v_i$) will be large. The growth rate $r_i$ along each pathway is modeled with modified Monod rate equations in which the rate is proportional to the intracellular concentration of a key enzyme $e_i$ controlling the $i$-th pathway:

$$r_1 = \mu_1 e_1 \frac{G}{K_1 + G}$$
$$r_2 = \mu_2 e_2 \frac{E}{K_2 + E} \frac{O}{K_{O_2} + O}$$
$$r_3 = \mu_3 e_3 \frac{G}{K_3 + G} \frac{O}{K_{O_3} + O}$$
where: \( G, E \) and \( O \) represent the concentrations of glucose, ethanol and dissolved oxygen, respectively; \( \mu_i \) are maximum growth rate constants; \( K_i \) and \( K_{oi} \) are saturation constants for the substrate and dissolved oxygen, respectively.

The cybernetic model equations presented in the original reference [42] contain several typographical errors. The corrected mass balances are written as:

\[
\frac{dX}{dt} = \left( \sum_i (r_i v_i) - D \right) X
\]
\[
\frac{dC}{dt} = \gamma_3 r_3 v_3 - \left( \gamma_1 r_1 v_1 + \gamma_2 r_2 v_2 \right) C - \sum_i (r_i v_i) C
\]
\[
\frac{dG}{dt} = D(G_0 - G) - \left( \frac{r_1 v_1}{Y_1} + \frac{r_3 v_3}{Y_3} \right) X - \phi_1 \left( C \frac{dX}{dt} + X \frac{dC}{dt} \right)
\]
\[
\frac{dE}{dt} = -DE + \left( \phi_1 \frac{r_1 v_1}{Y_1} - \frac{r_2 v_2}{Y_2} \right) X
\]
\[
\frac{dO}{dt} = k_L a (O^* - O) - \left( \phi_2 \frac{r_2 v_2}{Y_2} + \phi_3 \frac{r_3 v_3}{Y_3} \right) X
\]
\[
\frac{de_1}{dt} = \alpha u_1 \frac{G}{K_1 + G} - \left( \sum_i (r_i v_i) + \beta \right) e_1 + \alpha^*
\]
\[
\frac{de_2}{dt} = \alpha u_2 \frac{E}{K_2 + E} - \left( \sum_i (r_i v_i) + \beta \right) e_2 + \alpha^*
\]
\[
\frac{de_3}{dt} = \alpha u_3 \frac{G}{K_3 + G} - \left( \sum_i (r_i v_i) + \beta \right) e_3 + \alpha^*
\]

(2.4)

where: \( X \) and \( C \) are the cell mass concentration and intracellular storage carbohydrate mass fraction, respectively; \( D \) is the dilution rate; \( G_0 \) is the glucose feed concentration; \( k_L a \) is the dissolved oxygen mass transfer coefficient; \( O^* \) is the saturation concentration of oxygen; \( Y_i \) are yield coefficients; \( \phi_i \) and \( \gamma_i \) are stoichiometric coefficients for substrate and intracellular storage carbohydrate synthesis and consumption, respectively; \( \alpha \) and \( \beta \) are enzyme synthesis and decay rate constants,
respectively; and $\alpha^*$ is a constant parameter associated with constitutive enzyme synthesis. A more detailed description of the model can be found in [42]. It is worth noting that the cybernetic model does not generate sustained oscillations with the parameter values exactly as given in [42]. The parameter values used in the subsequent simulation and bifurcation studies are given in Table 2.3. The parameters are obtained from [42] with the exception that $\alpha^* = 0.03 \text{ g/g-h}.$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{\text{max}}$</td>
<td>h$^{-1}$</td>
<td>0.44, 0.19, 0.36</td>
</tr>
<tr>
<td>$K_i$</td>
<td>g/L</td>
<td>0.05, 0.01, 0.001</td>
</tr>
<tr>
<td>$Y_i$</td>
<td>g/g</td>
<td>0.16, 0.75, 0.60</td>
</tr>
<tr>
<td>$\phi_i$</td>
<td>g/g</td>
<td>0.403, 2.0, 1.0, 0.95</td>
</tr>
<tr>
<td>$\gamma_i$</td>
<td>g/g</td>
<td>10, 10, 0.8</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>g/g-h</td>
<td>0.3</td>
</tr>
<tr>
<td>$\alpha^*$</td>
<td>g/g-h</td>
<td>0.03</td>
</tr>
<tr>
<td>$\beta$</td>
<td>h$^{-1}$</td>
<td>0.7</td>
</tr>
<tr>
<td>$K_{O_2}$</td>
<td>mg/L</td>
<td>0.01</td>
</tr>
<tr>
<td>$K_{O_3}$</td>
<td>mg/L</td>
<td>2.2</td>
</tr>
<tr>
<td>$k_L a$</td>
<td>h$^{-1}$</td>
<td>225</td>
</tr>
<tr>
<td>$O^*$</td>
<td>mg/L</td>
<td>7.5</td>
</tr>
</tbody>
</table>

### 2.5.3 Results and Discussion

As mentioned previously, the cybernetic model does not produce sustained oscillations with the parameter values given exactly as in [42]. Because the value of the parameter $\alpha^*$ is not specified in the original reference, a primitive search was conducted to determine combinations of the parameters that support periodic solutions. The following combinations of $D$ and $G_0$ were found by dynamic simulation to produce sustained oscillations when $\alpha^* = 0.03 \text{ g/g-h}:
• $D = 0.16 \text{ h}^{-1}$ and $G_0 \in [9.5 \text{ g/L}, 10.5 \text{ g/L}].$

• $D = 0.14 \text{ h}^{-1}$ and $G_0 \in [9.7 \text{ g/L}, 12.3 \text{ g/L}].$

• $G_0 = 10 \text{ g/L}$ and $D \in [0.14 \text{ h}^{-1}, 0.16 \text{ h}^{-1}].$

• $G_0 = 12.3 \text{ g/L}$ and $D \in [0.118 \text{ h}^{-1}, 0.14 \text{ h}^{-1}].$

Clearly this is a very inefficient method for determining the range of parameter values. Bifurcation analysis is shown below to be a much more powerful tool for determining such information. It is worth noting that in the original reference [42] bifurcation analysis is used to determine the range of four unknown parameters ($\mu_3$, $K_3$, $K_{O_2}$, $K_{O_3}$) that support periodic solutions.

First the transient behavior of the model is studied via dynamic simulation. Figure 2.7 shows two simulation tests at high dilution rates. The cell mass concentration ($X$) is chosen as the output variable for the culture. Both simulations are initiated with the steady-state solution corresponding to $D = 0.165 \text{ h}^{-1}$ and $G_0 = 10 \text{ g/L}$. The feed glucose concentration is maintained at this value while the dilution rate is changed slightly. When the dilution rate is changed to $D = 0.1648 \text{ h}^{-1}$ the model moves to a new steady-state solution, while for $D = 0.1646 \text{ h}^{-1}$ the model exhibits sustained oscillations of a small amplitude. Figure 2.8 shows some simulation results at lower dilution rates and the same feed glucose concentration. The simulations are initiated with the steady-state solution for $D = 0.13 \text{ h}^{-1}$. When the dilution rate is changed to $D = 0.1366 \text{ h}^{-1}$, the model initially oscillates and
Figure 2.7: Dynamic simulation of the *Saccharomyces cerevisiae* reactor model at high dilution rates.

then reaches a new steady state at approximately 200 h. When $D = 0.1367 \text{ h}^{-1}$, the model exhibits sustained oscillations with a considerably larger amplitude than that shown in Figure 2.7. At $t = 200 \text{ h}$, the dilution rate of the latter simulation is reduced to $D = 0.1366 \text{ h}^{-1}$. While the amplitude is decreased, sustained oscillations are maintained even though the same dilution rate previously produced a steady-state solution.

These simulation results demonstrate the cybernetic model is capable of predicting the appearance and disappearance of sustained oscillation over a range of operating parameters. However, the results indicate significant differences between
experiment and simulation with respect to the oscillation amplitude at low and high dilution rates as well as the hysteresis behavior observed at low dilution rates. Therefore, bifurcation analysis is needed to investigate these issues. It is well known that continuous yeast bioreactors exhibit sustained oscillations only for a specific range of dilution rates [64]. This suggests that the dilution rate \( D \) is a reasonable choice for the bifurcation parameter. In this case, the feed glucose concentration \( G_0 \) is fixed at 10 g/L.

The one-parameter bifurcation diagram with \( D \) as the bifurcation parameter is shown in Figure 2.9 where the glucose concentration \( G \) is chosen as the output
variable. The solid lines represent stable steady-state and periodic solutions while the dashed lines represent unstable solutions. The middle branch between points 1–4 represents steady-state solutions for different dilution rates. The upper branch defined by points 1, 5 and 2 represents the largest $G$ value obtained for the periodic solution at the given dilution rate. The lower branch consisting of points 1, 6 and 2 defines the lower $G$ limit of the periodic solutions. There is a single stable steady-state solution at low dilution rates. When $D$ is increased to $0.13664\text{ h}^{-1}$ (point 1), a subcritical Hopf bifurcation occurs and the steady-state solution becomes unstable. The periodic solution generated initially is unstable and bends back toward low dilution rates until a fold bifurcation occurs at $D = 0.13654\text{ h}^{-1}$ (point 5). Past this point the periodic solution is stable.

For a wide range of dilution rates, the stable periodic solution coexists with an unstable steady-state solution. When $D$ is increased to $0.16496\text{ h}^{-1}$ (point 2), a supercritical Hopf bifurcation occurs which causes the periodic solution to disappear and the steady-state solution to regain its stability. The steady-state solution maintains its stability until a branching point [46] at $D = 0.20901\text{ h}^{-1}$ (point 3) is reached. Then the steady-state solution turns back in the direction of decreasing dilution rate and become unstable. Another fold bifurcation occurs at $D = 0.18341\text{ h}^{-1}$ (point 4) where the steady-state solution becomes stable once again. Although not shown, higher dilution rates will cause the steady state to lose stability and the wash-out steady state to become stable.
Figure 2.9: One-parameter bifurcation diagram of the *Saccharomyces cerevisiae* reactor model with $D$ as the bifurcation parameter and $G_0 = 10 \text{ g/L}$. 

The bifurcation structure shown in Figure 2.9 provides a concise explanation of the previous dynamic simulation results. The appearance and disappearance of sustained oscillations is attributable to two Hopf bifurcations, as also was noted by Jones *et al.* [41]. However, considerably more information can be extracted from the bifurcation diagram:

- The first Hopf bifurcation is subcritical and the associated periodic solution undergoes a fold bifurcation. Consequently, there exists a very small range of dilution rates $[0.13654 \text{ h}^{-1}, 0.13664 \text{ h}^{-1}]$ where there coexists a stable steady state, an unstable periodic solution and a stable periodic solution. For these parameter values, the range is too small for the multiple stable solutions to
be observed experimentally. Nevertheless, the subcritical form of the bifurcation agrees with the experimental observation and the simulation results (Figure 2.8) that sustained oscillations at lower dilution rates have a large amplitude once established.

- The second Hopf bifurcation point is supercritical. Therefore, the periodic solution that emanates from this bifurcation has very small amplitude. This explains the dynamic simulation results in Figure 2.7. There is no experimental studies in the literature that demonstrate a difference between the oscillation amplitudes that arise from bifurcations at low and high dilution rates. However, this model prediction could be investigated experimentally.

- There is hysteresis for dilution rates in the range of $D \in [0.18341 \text{ h}^{-1}, 0.20901 \text{ h}^{-1}]$ due to the presence of multiple stable steady-state solutions. Hysteresis could have an adverse effect on the reactor operability. It appears that such behavior has not been reported by other theoreticians or experimentalists; further experimental studies are required to test this model prediction.

Additional knowledge can be obtained by investigating the bifurcation behavior with respect to two parameters simultaneously. Figure 2.10 shows the two-parameter bifurcation diagram with dilution rate ($D$) and feed glucose concentration ($G_o$) as the bifurcation parameters. The figure depicts the locus of the Hopf bifurcation points where HB1 and HB2 refer to points 1 and 2, respectively, in Figure 2.9. The region contained inside these two loci represents the operating space that supports
sustained oscillations. Unlike the two-parameter bifurcation diagram for the *Zymomonas mobilis* model in Figure 2.6, the parameter region is not closed and there is a multiplicity phenomena as shown in the inset. The locus for HB2 is monotonic, while the locus for HB1 has an “S” shape. This implies that for a certain region of operating conditions, HB1 bifurcates into multiple bifurcation points at a fixed $G_0$ or a fixed $D$. Therefore, a one-parameter bifurcation analysis with the other parameter fixed at an appropriate value will yield two or three bifurcation points.

Figure 2.10: Two-parameter bifurcation diagram of the *Saccharomyces cerevisiae* reactor model with $D$ and $G_0$ as the bifurcation parameters.
which originate from point 1 in Figure 2.9. As shown in Figure 2.10, there is a single bifurcation point for $G_0 = 10 \text{ g/L}$.

The one-parameter bifurcation diagram with $D$ as the bifurcation parameter and $G_0 = 9.25 \text{ g/L}$ is shown in Figure 2.11. Similar to Figure 2.9, the branch between points 1–4 represents a steady-state solution contained within a periodic solution appearing from the subcritical Hopf bifurcation point (point 1) and disappearing at the supercritical Hopf bifurcation (point 4). The periodic solution emanating from point 1 is unstable until the fold bifurcation (point 6).

Figure 2.11: One-parameter bifurcation diagram of the *Saccharomyces cerevisiae* reactor model with $D$ as the bifurcation parameter and $G_0 = 9.25 \text{ g/L}$.
Unlike Figure 2.9, there now exists an unstable periodic solution within the large-amplitude stable periodic solution. The unstable periodic solution emanates from a supercritical Hopf bifurcation (point 2) and ends at another supercritical Hopf bifurcation (point 3). Within this periodic solution, the steady-state solution regains its stability. Therefore, within the range $D \in [0.15100 \text{ h}^{-1}, 0.15883 \text{ h}^{-1}]$ there is a stable steady-state solution, an unstable periodic solution and a stable periodic solution. The cybernetic model can predict the coexistence of a stable steady-state solution and a stable periodic solution over a meaningful range of dilution rates. It is important to emphasize that the existence of multiple attractors would be quite difficult to observe using dynamic simulation alone. This again supports that bifurcation analysis is a very powerful tool for model analysis and validation.

The behavior shown in Figure 2.11 is particularly interesting as multiple attractors have been observed experimentally in continuous cultures of *Saccharomyces cerevisiae* [85]. Figure 2.12 shows the results of an experiment [81] designed to examine this phenomenon. The evolved carbon dioxide signal is used as a representative output variable for the culture. The experiment starts with oscillatory dynamics that are obtained by switching the culture from batch to continuous operation. At $t = 20$ h the dilution rate is slowly ramped down over a 24 hour period until the oscillations disappear. The stationary state is preserved for two days while the dilution rate is maintained at the low value. At $t = 92$ h the dilution rate is slowly ramped up at the same rate as was used for the negative ramp. Oscillations
Figure 2.12: Experimentally observed transient behavior in a continuous culture of *Saccharomyces cerevisiae* for ramp changes in the dilution rate [81].

are not observed when the dilution rate is maintained at the high value despite the fact that a slightly lower dilution rate produced oscillations at the beginning of the experiment. An enlarged view of the dynamic behavior during the first and last parts of the experiment is shown in Figure 2.13. The upper plot shows the large amplitude oscillations obtained at the beginning of the experiment, while the lower plot shows the stationary response observed at the end of the experiment. The bifurcation analysis in Figure 2.11 suggests that the cybernetic model can predict the existence of such multiple attractors.

A one-parameter bifurcation diagram with $D$ as the bifurcation parameter and $G_0$ fixed at 8.7 g/L is presented in Figure 2.14. As shown in Figure 2.10, there
Figure 2.13: Experimentally observed multiple attractors in a continuous culture of *Saccharomyces cerevisiae*.

are two bifurcation points that emanate from point 1 in Figure 2.9. In this case, both of the Hopf bifurcations are subcritical. The range of dilution rates that support periodic solution is quite small, and sustained oscillations might be difficult to observe experimentally. The bifurcation structure for dilution rates outside the region shown is very similar to that in Figure 2.9 and has been omitted.

2.6 Summary and Conclusions

The dynamic behavior of three continuous bioreactor models that exhibit complex steady-state and transient behavior is studied. Bifurcation analysis is shown to
Figure 2.14: One-parameter bifurcation diagram of the *Saccharomyces cerevisiae* reactor model with $D$ as the bifurcation parameter and $G_0 = 8.75$ g/L.

provide a more complete picture of model behavior than is possible with dynamic simulation alone. The determination of bifurcation points when the qualitative model behavior changes and the characterization of the range of parameter values that supports certain behaviors is valuable information for model validation. Several important characteristics of the three models studied that are not observed in previous simulation studies are revealed through bifurcation analysis. These characteristics include lack of model robustness to small parameter variations, apparent inconsistencies between model structure and experimental data, and the coexistence of multiple stable solutions under the same operating conditions. These case
studies suggests that bifurcation analysis is a very powerful tool for analyzing low-dimensional bioreactor models and should be used in conjunction with dynamic simulation for model validation.
Chapter 3

Cell Population Models for Bifurcation Analysis and Nonlinear Control of Continuous Yeast Bioreactors

3.1 Introduction

Saccharomyces cerevisiae (baker’s yeast) is an important microorganism in the brewing, baking, food manufacturing and genetic engineering industries. Many investigators have shown that continuous cultures of Saccharomyces cerevisiae exhibit sustained oscillations in glucose limited environments under aerobic growth conditions [12, 56, 62, 64, 68, 78, 80]. A precise characterization of the environmental conditions that support oscillatory dynamics has not been developed because oscillations often appear and disappear without any measurable change in external inputs such as nutrient flow and concentration. The underlying cellular mechanisms that cause oscillatory yeast dynamics are controversial and have been a subject of three decades of intensive research. Understanding and controlling this dynamic behavior would lead to important advances in yeast production processes and could provide key insights into the cellular behavior of more complex eucaryotic cells present in plants and animals.
A large number of transient models have been proposed to explain the sustained oscillations observed in continuous cultures of baker’s yeast. Existing models can be classified into three categories: structured and unsegregated [14, 31, 42]; unstructured and segregated [11, 21, 34, 36]; and structured and segregated [15, 76, 77]. Structured models account for various chemical components and their interactions within the cell. By contrast unstructured models are based on the simplifying assumption that detailed modeling of intracellular behavior is not essential to describe cell growth. Segregated models account for differences between individual cells in terms of properties such as cell mass or cell age. Unsegregated models are based on the simplifying assumption that individual cells have identical physical and chemical properties.

Oscillatory dynamics produced by structured, unsegregated models are a direct result of cell metabolism incorporated into the model. For example in the cybernetic model proposed in [42] oscillations arise from competition between three metabolic pathways: glucose fermentation, glucose oxidation and ethanol oxidation. A shortcoming of purely metabolic models is that they cannot adequately explain cell cycle synchronization that leads to the formation of distinct cell subpopulations. It is well known that synchronization plays a critical role in the establishment and maintenance of oscillatory yeast dynamics [62, 68, 70].

In unstructured, segregated models oscillations arise due to cell population dynamics rather than cell metabolism. The key feature of these models is a population
balance equation (PBE) that describes the time evolution of the cell age or cell mass distribution. Sustained oscillations can be generated by coupling the PBE and the extracellular environment to establish a synchrony induction mechanism [33]. While they are capable of predicting cell cycle synchrony, these models cannot capture the interplay between cell metabolism and oscillatory dynamics due to their unstructured nature [42]. Structured, segregated models have been developed to address the limitations of the simpler models described above. The most sophisticated model of this type is presented in [77]. A discretized form of the cell mass distribution is combined with a metabolic model that accounts for basic intracellular variables such as storage carbohydrates. The model is capable of producing sustained oscillations with periods comparable to those observed experimentally. However the model is not well suited for control applications due to its complexity.

Zhu et al. [89] have shown that an unstructured, segregated model in which the PBE for the cell mass distribution is coupled to the mass balance of the rate limiting substrate can predict sustained oscillations in continuous yeast bioreactors. However, the underlying mechanism that causes the model to produce periodic solutions is not investigated. Since the appearance and disappearance of periodic solutions of a nonlinear system is attributable to bifurcation phenomena, a detailed bifurcation analysis will provide key insights into the PBE model structure and may motivate further experimental studies aimed at verifying the model predictions. Such a bifurcation study is presented in this chapter.
Zhu et al. have used the PBE model to develop a linear model predictive control (LMPC) strategy for attenuating and inducing sustained oscillations in continuous yeast bioreactors [89]. The linear controller design model is obtained by linearizing and temporally discretizing the ordinary differential equations derived from spatial discretization of the PBE model. The resulting linear state-space model has been used to develop LMPC controllers that regulate the discretized cell number distribution by manipulating the dilution rate and feed substrate concentration. While some preliminary simulation tests have been encouraging, the LMPC strategy suffers from several potential disadvantages including: (i) the controller design model is linear even though bifurcations are a nonlinear phenomenon; (ii) the cell mass distribution is assumed to be measured or reconstructed from particle size measurements; (iii) the resulting control problem is highly non-square (2 inputs, 14 outputs); and (iv) direct control of the cell distribution is complex and often unnecessary as typical end products are metabolites rather than the yeast itself. In this chapter nonlinear controllers that are easier to implement were developed to provide improved closed-loop performance.

The remainder of this chapter is organized as follows. Existing experimental and modeling work is briefly reviewed in Section 3.2. The application of bifurcation analysis techniques to the cell population model is discussed in Section 3.3. Nonlinear controller design based on a moment representation of the PBE model
and closed-loop simulation results are presented in Section 3.4. A summary and conclusion is presented in Section 3.5.

3.2 Cell Population Model for Continuous Yeast Bioreactors

3.2.1 Experimental Data

There is a large body of experimental data of transient behavior of intracellular and extracellular variables during sustained oscillations of continuous yeast cultures. It is well known that yeast cultures exhibit oscillations only for a specific range of dilution rates [64]. Stationary solutions are observed for dilution rates below and above this range. Zamamiri et al. have conducted additional experiments to explore these oscillatory dynamics. Batch and continuous culture experiments were performed using a Bioflo 3000 fermenter (New Brunswick) with a working volume of 1.0 L interfaced to a personal computer with the necessary software for data collection and basic regulatory control functions. Details on the medium preparation and experimental protocol are available in [81].

One set of experiments was designed to test the hypothesis that a stable steady state and a stable periodic solution can coexist at the same operating conditions. A representative set of results for a glucose feed concentration of 30 g/L is shown in Figure 2.12. The evolved carbon dioxide signal is selected as a representative output variable for the culture. The experiment starts with oscillatory dynamics that are obtained by switching the culture from batch to continuous operation. At $t = 20$ hr
the dilution rate is slowly ramped down over a 24 hour period until the oscillations disappear. The objective is to select the ramp rate to be sufficiently small that the fermenter remains in a quasi-steady state condition. The stationary state is preserved for two days while the dilution rate is maintained at the low dilution rate. Then at \( t = 92 \text{ hr} \) the dilution rate is slowly ramped up at the same rate as used for the negative ramp. No significant oscillations are observed when the dilution rate is maintained at this high value despite the fact that a slightly lower dilution rate produced oscillations at the beginning of the experiment.

An enlarged view of the dynamic behavior during the first and last parts of the experiment is shown in Figure 2.13. The upper plot shows the large amplitude oscillations obtained at the beginning of the experiment. The lower plot shows the response observed at the end of the experiment. The evolved \( \text{CO}_2 \) signal in the lower plot appears to represent a stationary solution. This suggests the coexistence of stable steady-state and stable periodic solutions under the same operating conditions. When examined more carefully the signal in the lower plot appears to contain a periodic component that is not solely attributable to measurement noise. It is possible that this solution actually is unstable but appears to be stable due to the relatively short duration of the test. Consequently the long-term stability of this “stationary” solution is questionable. Nevertheless a candidate yeast model should be capable of capturing this apparent hysteresis behavior.
3.2.2 Yeast Cell Population Model

Hjortsø and Nielsen [35] propose a conceptual model for budding yeast cultures which can be used to predict operating conditions under which periodic solutions exist. The model couples the PBE for the cell age distribution to the substrate mass balance. Cell age is used as the internal coordinate because this choice avoids the complications associated with modeling individual cell growth kinetics. Zhu et al. [89] present an enhanced PBE model that provides a more realistic description of the yeast cell cycle dynamics. The proposed modifications include: (i) the use of cell mass instead of cell age as the internal coordinate to facilitate real-time measurement of the cell number distribution; (ii) probabilistic descriptions of cell transition and division rather than the assumption of discrete control points; and (iii) coupling of the PBE to the substrate balance through a filtered value of the substrate concentration rather than a delayed value. The simplified cell cycle model from which the PBE model is derived is illustrated in Figure 3.1. A daughter cell grows until it reaches a critical mass called the transient mass \( m_t^* \). At this point the cell is called a mother cell. All further growth occurs in the bud attached to the mother cell. At a critical mass called the division mass \( m_d^* \) the mother cell and the bud divide to produce a newborn daughter cell and a newborn mother cell. The critical masses that characterize the cell cycle depend on the extracellular conditions as discussed below.
The PBE model equations are briefly present below to facilitate the subsequent development. Additional details are available in [89]. The PBE has the form:

\[
\frac{\partial W(m, t)}{\partial t} + \frac{\partial [k(S')W(m, t)]}{\partial m} = \int_0^\infty 2p(m, m')\Gamma(m', S')W(m', t)dm' - [D + \Gamma(m)]W(m, t)
\]

(3.1)

where: \(m\) is the cell mass; \(W(m, t)\) is the cell number density; \(k(S')\) is the single cell growth rate; \(S'\) is the filtered substrate concentration (defined below); \(p(m, m')\) is the newborn cell probability function; \(\Gamma(m, S')\) is the division intensity function; and \(D\) is the dilution rate. The initial cell distribution is denoted \(W(m, 0)\) and the boundary condition is \(W(0, t) = 0\). The zeroth moment of the cell number density represents the total number of cells per unit volume and is defined as: \(m_0(t) = \)
\[ f_0^\infty W(m, t) dm. \] The differential equation describing the evolution of the zeroth moment is easily derived [45]:

\[
\frac{dm_0}{dt} = -Dm_0 + \int_0^\infty \Gamma(m, S') W(m, t) dm
\]  \( (3.2) \)

The division intensity function is modeled as:

\[
\Gamma(m, S') = \begin{cases} 
0 & m \leq m^*_i + m_a \\
\gamma \exp[-\epsilon(m - m^*_d)^2] & m \in [m^*_i + m_a, m^*_d] \\
\gamma & m \geq m^*_d 
\end{cases}
\]  \( (3.3) \)

where \( m^*_i \) is the transition mass, \( m_a \) is the additional mass that mother cells must gain before division is possible, \( \epsilon \) and \( \gamma \) are constant parameters and \( m^*_d \) is the division mass at which the division intensity function reaches its maximum value \( \gamma \).

The newborn cell probability function \( p(m, m') \) is chosen as:

\[
p(m, m') = A \exp[\beta (m - m^*_i)^2] + A \exp[\beta (m' - m^*_i + m_i^*)^2]
\]  \( (3.4) \)

when \( m < m' \) and \( m' > m^*_i + m_a \); the function is identically zero otherwise. Here \( A \) and \( \beta \) are constant parameters. This function yields two Gaussian peaks in the cell number distribution, one centered at the transition mass \( m^*_i \) (which corresponds to mother cells) and one centered at a location in the mass domain that is determined by mass conservation (which corresponds to daughter cells).
As suggested by available experimental data, the transition mass \( m^*_t \) and the division mass \( m^*_d \) are modeled as increasing functions of the substrate concentration:

\[
m^*_t(S') = \begin{cases} 
m_{t0} + K_t(S_l - S_h) & S' < S_l \\
m_{t0} + K_t(S' - S_h) & S' \in [S_l, S_h] \\
m_{t0} & S' > S_h
\end{cases}
\]

\[
m^*_d(S') = \begin{cases} 
m_{d0} + K_d(S_l - S_h) & S' < S_l \\
m_{d0} + K_d(S' - S_h) & S' \in [S_l, S_h] \\
m_{d0} & S' > S_h
\end{cases}
\]

(3.5)

where \( S_l, S_h, m_{t0}, m_{d0}, K_t \) and \( K_d \) are constant parameters. The substrate mass balance is:

\[
\frac{dS}{dt} = D(S_f - S) - \int_0^\infty \frac{k(S')}{Y} W(m, t) dm
\]

(3.7)

where \( Y \) is a constant cell mass yield coefficient. The single cell growth rate is assumed to follow Monod kinetics:

\[
k(S') = \frac{\mu_m S'}{K_m + S'}
\]

(3.8)

where the maximum growth rate \( \mu_m \) and the saturation parameter \( K_m \) are constants.

The filtered substrate concentration is generated as:

\[
\frac{dS'}{dt} = \alpha(S - S')
\]

(3.9)
where the constant parameter $\alpha$ indicates how fast cells respond to environmental changes.

The parameters used for model simulation are listed in Table 3.1. It is important to note that only a few of the parameter values (e.g. single cell growth rate kinetics) are available in the literature. Consequently the unknown parameters are chosen heuristically to yield reasonable bioreactor operating conditions and experimentally observed dynamic behavior. Possible future research could include the estimation of unknown model parameters from experimental data generated in LSU chemical engineering laboratory.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma$</td>
<td>200 hr$^{-1}$</td>
<td>$\epsilon$</td>
<td>$5 \times 10^{22}$ g$^{-2}$</td>
</tr>
<tr>
<td>$A$</td>
<td>$\sqrt{25/\pi}$ g$^{-1}$</td>
<td>$\beta$</td>
<td>$100 \times 10^{22}$ g$^{-2}$</td>
</tr>
<tr>
<td>$S_l$</td>
<td>0.1 g/L</td>
<td>$S_h$</td>
<td>2 g/L</td>
</tr>
<tr>
<td>$K_l$</td>
<td>$0.01 \times 10^{-11}$ g/g·L</td>
<td>$K_d$</td>
<td>$2 \times 10^{-11}$ g/g·L</td>
</tr>
<tr>
<td>$m_{io}$</td>
<td>$6 \times 10^{-11}$ g</td>
<td>$m_{do}$</td>
<td>$11 \times 10^{-11}$ g</td>
</tr>
<tr>
<td>$m_{max}$</td>
<td>$12 \times 10^{-11}$ g</td>
<td>$m_a$</td>
<td>$1 \times 10^{-11}$ g</td>
</tr>
<tr>
<td>$Y$</td>
<td>0.4 g/g</td>
<td>$\mu_m$</td>
<td>$5 \times 10^{-10}$ g/hr</td>
</tr>
<tr>
<td>$K_m$</td>
<td>25 g/L</td>
<td>$\alpha$</td>
<td>20 hr$^{-1}$</td>
</tr>
<tr>
<td>$D$</td>
<td>0.25 hr$^{-1}$</td>
<td>$S_f$</td>
<td>25 g/L</td>
</tr>
</tbody>
</table>

The PBE model is comprised of a coupled set of nonlinear algebraic, ordinary differential and integro-partial differential equations [79, 51, 54]. Analytical solution of most models is possible only under very restrictive assumptions [35, 36]. A variety of numerical solution techniques based on finite difference, weighted residuals and orthogonal collocation methods have been developed for such PBE models. The
objective of such methods is to convert the PBE into a coupled set of ODEs with
time as the independent variable. The resulting ODE model can be solved using
standard numerical integration procedures.

In this chapter the solution technique based on orthogonal collocation on finite
elements proposed by Zhu et al. [89] is used. The mass domain is discretized into a
number of finite elements, each of which contains several collocation points where the
PBE is approximated by an ODE. Integral terms are approximated using Gaussian
quadrature. The state vector of the resulting nonlinear ODE model consists of the
cell number density at each collocation point, as well as the substrate and filtered
substrate concentrations. In this study, the discretization scheme uses 12 finite
element and 8 internal collocation points on each finite element to yield a total of
111 state variables.

The dynamic response of the cell population model to a dilution rate ramp test
similar to that performed experimentally (see Figure 1) is shown in Figure 3.2.
Here the substrate concentration ($S$) is used as the output variable since the PBE
model does not account for the evolved CO$_2$ concentration. The dilution rate is
ramped down from the oscillatory region ($D = 0.21$ h$^{-1}$) to the non-oscillatory
region ($D = 0.18$ h$^{-1}$) and then ramped back up to the original dilution rate. The
simulated oscillation period (2.5 h) is very close to the value observed experimentally
(2.2 h). However the simulated negative ramp leads to very slow damping of the
oscillations as compared to that observed experimentally. On the other hand, the
simulated positive ramp produces oscillations of a much smaller amplitude than those observed during the initial portion of the test. It is difficult to ascertain if the model predictions are consistent with experimental data due to the questions surrounding the stability of the steady-state solution in the oscillatory range. A more detailed analysis of the PBE model is possible using bifurcation analysis.

3.3 Bifurcation Analysis

Bifurcation analysis is a powerful tool for studying the dynamic behaviors of nonlinear models. It is desirable to characterize the bifurcations that lead to the appearance and disappearance of periodic solutions in the yeast cell population model.
This allows a more insightful comparison to experimental data than is possible with
dynamic simulations alone. The vast majority of bifurcation theory is developed for
nonlinear ODE systems [46]. Consequently it is necessary to spatially discretize the
PBE model prior to analysis. Given the complexity of the resulting model, numerical
bifurcation techniques must be pursued as theoretical analysis is not tractable.

### 3.3.1 Computational Methods

It is well known that yeast cultures exhibit sustained oscillations only for a specific
range of dilution rates [64]. This suggests that the dilution rate is an appropriate
choice for the bifurcation parameter. As the dilution rate is increased the PBE model
should exhibit two bifurcations corresponding to the appearance and disappearance
of sustained oscillations.

The first task is to determine the local stability of steady-state solutions for
different values of the bifurcation parameter. Steady-state solutions can be obtained
by solving the steady-state version of the spatially discretized PBE model using
nonlinear algebraic equation solvers based on the Newton-Raphson method. In
this study the MATLAB routine `fsolve` is used. The Jacobian matrix is generated
analytically by linearizing the nonlinear model about the desired steady state. Local
stability of the steady state is determined by computing the eigenvalues of the
Jacobian using the MATLAB routine `eig`. A dilution rate where one or more
eigenvalues cross the imaginary axis is known as a bifurcation point [46].
Locating periodic solutions and determining their stability is a much more difficult problem. A stable periodic solution can be located by dynamic simulation if the initial condition is in the domain of attraction. In addition to being restricted to stable solutions, this approach is time consuming and subject to misinterpretation if the oscillatory solution exhibits slow divergence from periodicity. A more efficient and reliable alternative is to use the shooting method to locate both stable and unstable periodic solutions [46]. This approach requires a good initial guess of the state variables and the oscillation period corresponding to the periodic solution. For stable periodic solutions, the initial point usually is obtained by dynamic simulation.

The shooting method involves the following iterative calculation procedure. First the model is integrated over one oscillation period. The difference between the initial and ending points is used to adjust the values of the state vector and period. During each of these Newton iterations, one preselected state variable is fixed to provide a basis for determining the period. This is known as the pinning condition. The iterations continue until a convergence criterion is satisfied. The procedure is repeated for other dilution rates using a process known as continuation [46].

The shooting method effectively transforms the $n$-dimensional continuous-time system into an $(n-1)$-dimensional discrete-time system. The Poincaré map transforms a periodic solution of the continuous-time system to a fixed point on the $(n-1)$-dimensional hyperplane [46]. Stability of the periodic solution is determined by examining the magnitude of the Floquet multipliers which characterize the sta-
bility of the linearized Poincaré map. There always is a single Floquet multiplier of unity magnitude due to the translational invariance of the solution in time. The periodic solution is stable if the remaining $n-1$ Floquet multipliers are inside the unit circle.

A complete investigation of the PBE model dynamics requires a more detailed bifurcation analysis than is possible using the techniques described above. Several continuation packages have been developed for detailed bifurcation analysis of low-dimensional problems. Unfortunately the dimensionality of the discretized cell population model ($x \in \mathcal{R}^{111}$) precludes the use of such general purpose continuation packages. Therefore a continuation code obtained from Prof. Ioannis Kevrekidis (Princeton) specifically designed for locating limit cycles of high-dimensional systems is utilized in this study. The ODE solver ODESSA is used for numerical integration.

### 3.3.2 Results

A bifurcation diagram for the spatially discretized cell population model is shown in Figure 3.3 where the dilution rate ($D$) is the bifurcation parameter and the substrate concentration ($S$) is chosen as a representative output variable. The feed substrate concentration ($S_f$) is held constant at 25 g/L. The model possesses a single stable steady-state solution (+) at low dilution rates. As the dilution rate is increased a bifurcation occurs at $D = 0.205 \text{ h}^{-1}$ which is characterized by the steady-state solution becoming unstable (o) and the appearance of a stable periodic solution with
oscillations of the magnitude indicated (*). The spectrum of the Jacobian matrix shows that a pair of eigenvalues cross from the left-half plane (LHP) to the right-half plane (RHP) at this point. Therefore this is a supercritical Hopf bifurcation [46]. For a large range of dilution rates the stable periodic solution coexists with the unstable steady-state solution. As the dilution rate is increased to $D = 0.285 \text{ h}^{-1}$, the periodic solution disappears and the steady-state solution regains stability. Analysis of the Jacobian matrix shows that the RHP eigenvalues cross back into LHP in a second supercritical Hopf bifurcation.

![Figure 3.3: Bifurcation diagram of the cell population balance model with dilution rate as the bifurcation parameter.](image-url)
Figure 3.4 shows the spectrum of the discretized cell population model at a steady-state solution corresponding to a dilution rate \( D = 0.25 \text{ h}^{-1} \) located in the middle of the oscillatory region. The model has a total of 111 eigenvalues due to the number of collocation points used. Many of the eigenvalues \( (\lambda_i) \) are located near the imaginary axis. In particular there is a complex conjugate pair of eigenvalues located in the RHP (see inset). This shows that the steady state is unstable and suggests the existence of a stable periodic solution. Because the RHP eigenvalues are located very close to the imaginary axis, transitions between stable steady-state and stable periodic solutions are quite slow as shown in Figure 3.2. While this property is not desirable for the negative ramp change, it provides a plausible mechanism for the apparent hysteresis behavior observed experimentally in Figure 2.12 since the “stationary” solution actually may represent an oscillatory solution of very small amplitude. Experiments are currently being conducted to definitively determine the long-term stability of the steady state.

Since the feed substrate concentration \( (S_f) \) also is a bioreactor input, it is useful to investigate the dynamic behavior of the model with respect to this variable. Figure 3.5 shows the bifurcation diagram of the discretized PBE model where \( S_f \) is the bifurcation parameter and the substrate concentration \( S \) is chosen as the output variable. The dilution rate is fixed at \( 0.25 \text{ h}^{-1} \). For small feed substrate concentration \( (S_f < 12.7 \text{ g/L}) \) there is only a single stable steady-state solution. For \( S_f \) values above this limit the steady-state solution becomes unstable and a
Figure 3.4: Spectrum of the cell population model.

stable periodic solution is observed. This transition is attributable to a supercritical Hopf bifurcation.

In Zhang et al., a bifurcation analysis of the cell population model has suggested the steady-state solution is stable at dilution rates that support sustained oscillations [86]. A mechanism involving fold bifurcations of limit cycles was proposed to explain this behavior. In that work the Jacobian matrices were calculated using a finite difference approximation. While all the eigenvalues of the approximate Jacobian matrix are in the left-half plane, a complex conjugate pair of eigenvalues
Figure 3.5: Bifurcation diagram of the cell population balance model with feed substrate concentration as the bifurcation parameter.

is located very close to the imaginary axis. It is later found that the calculation of the approximate Jacobian is numerically ill-conditioned and the associated stability results are not reliable. In this chapter the Jacobian matrices are calculated analytically from the discretized PBE model and the bifurcation results have been verified with open-loop simulations using the ODE solver ODESSA. Therefore the bifurcation diagrams in Figure 3.3 and Figure 3.5 are indeed correct or at least more reliable.
3.4 Nonlinear Control

Control objectives for oscillating yeast cultures can include the attenuation and/or the stabilization of periodic solutions. Clearly the attenuation of undesirable oscillations will improve bioreactor operability under normal conditions. Oscillation induction may be desirable in certain situation; e.g. to increase the production of key metabolites produced preferentially during part of the cell cycle. In this work only the oscillation attenuation problem will be investigated. The bifurcation diagrams in Figures 3.3 and 3.5 show that the PBE model has a stable periodic solution and an unstable steady-state solution over a wide range of operating conditions. The goal is to modify the bifurcation structure such that periodic solutions are rendered unstable under feedback and the desired steady-state solution become globally asymptotically stable.

Ideally this would be achieved through the application of bifurcation-theoretic control techniques [17]. Unfortunately the complexity of the spatially discretized PBE makes this approach intractable. Zhu et al. [89] have developed a linear model predictive control strategy for oscillation attenuation of yeast cultures. As discussed in Section 1 this approach has several potential disadvantages, most notably the use of a linear model for the controller design. Input-output linearization [38] is a good candidate for this problem since it allows the use of a nonlinear model yet the controller is simple to design and implement. Kurtz et al. [45] have successfully
used this approach for the attenuation and induction of oscillation in binary fission cultures.

3.4.1 Controller Design Issues

The cell population model has two variables that may serve as manipulated inputs: the dilution rate ($D$) and the feed substrate concentration ($S_f$). It is not possible to achieve an arbitrary cell number distribution with these two inputs [89]. Instead only the cell number concentration ($m_0$) and/or the substrate concentration ($S$) will be controlled. First single-input, single-output (SISO) controllers will be designed, and evaluated. Then a multiple-input, multiple-output (MIMO) controller will be designed and the closed-loop results obtained with the SISO and MIMO controllers will be compared.

Given the two manipulated inputs ($D, S_f$) available and the two controlled outputs ($m_0, S$) chosen, there are a total of four candidate input/output pairings for nonlinear controller design. Note that the ODE (3.2) for $m_0$ does not include $S_f$ as an input. This input/output pair has a relative degree of two since $S_f$ affects $m_0$ through the substrate concentration ($S$) equation. To avoid differentiation of the integral in the $m_0$ equation, the $S_f/m_0$ pair is not used for controller design. Consequently the input/output pairs considered are $D/m_0$, $D/S$ and $S_f/S$. Using equipment in LSU Chemical Engineering department laboratory, $m_0$ and $S$ can be measured every 10–15 minutes. This frequency is not sufficient for satisfactory performance of the nonlinear controllers. However state-of-the-art measurement
technologies [75] are available that allow the 3 minute sampling time assumed in this study.

3.4.2 Results and Discussion

First the $D/m_0$ pair is considered for nonlinear controller design. The input/output linearizing control law is synthesized directly from the zeroth moment equation (3.2):

$$D = \frac{v - \int_0^\infty \Gamma(m, S') W(m, t) dm}{-m_0}$$  (3.10)

The integral term in this equation represents the growth rate of the cell number concentration due to cell division and can be inferred from common on-line measurements [25]. This integral is assumed to be known in this study. The input $v$ of the feedback linearized system is chosen to place the closed-loop poles and to include integral action for offset error tracking of the setpoint $m_0^*$:

$$v = \dot{m}_0^* - \alpha_1 m_0 + \alpha_0 \int_0^t (m_0^* - m_0) d\tau$$  (3.11)

where the tuning parameters $\alpha_0$ and $\alpha_1$ are chosen such that $s^2 + \alpha_1 s + \alpha_0$ is a Hurwitz polynomial. The second term in the right hand side of (3.11) is chosen as $-\alpha_1 m_0$ instead of $\alpha_1 (m_0^* - m_0)$ to eliminate overshoot in the closed-loop response. With simple algebraic manipulations it is easy to show that this control law yields the following closed-loop transfer function:
\[
g_d(s) = \frac{m_0(s)}{m_0^*(s)} = \frac{\alpha_0}{s^2 + \alpha_1 s + \alpha_0}
\]  

(3.12)

The desired closed-loop response is obtained by appropriate choice of the controller tuning parameters \((\alpha_0, \alpha_1)\).

The performance of the \(D/m_0\) feedback linearizing controller with \(\alpha_1 = 1 \text{ hr}^{-1}\) and \(\alpha_0 = 0.25 \text{ hr}^{-2}\) is shown in Figure 3.6. These tuning parameters yield a critically damped second-order response with a time constant of 2 hr. The open-loop response (---) corresponds to a stable periodic solution for \(D = 0.25 \text{ hr}^{-1}\) and \(S_f = 25 \text{ g/L}\). The controller is turned on at \(t = 4 \text{ hr}\) yielding the closed-loop response (—) shown. The ideal response corresponding to the closed-loop transfer function (3.12) also is shown (⋯). The setpoint \(m_0^* = 1.55 \times 10^{11} \text{ cells/L}\) corresponds to the total cell concentration for the nominal operating conditions in Table 1. The corresponding steady-state value for the substrate concentration \((S)\) is 0.8 g/L. The controlled output \(m_0\) closely follows the reference trajectory and the oscillations are completely eliminated. Deviations from the ideal closed-loop response are due to sampling. In addition oscillations in the uncontrolled output \(S\) are effectively damped and this variable is driven to its steady-state value.

The second controller is designed using the \(D/S\) pair. The input/output linearizing control law is synthesized directly from the substrate equation (3.7):
Figure 3.6: Feedback linearizing control using the $D/m_0$ pairing.

$$D = v + \int_0^\infty \frac{\kappa(s')}{Y} W(m, t) dm = v + \frac{\kappa(s')}{Y} m_0$$

(3.13)

where the input $v$ is designed as before:

$$v = \dot{S}^* - \alpha_1 S + \alpha_0 \int_0^t (S^* - S)d\tau$$

(3.14)

The third controller is designed using the $S_f/S$ pair. The input/output linearizing control law is derived from (3.7):
Figure 3.7: Feedback linearizing control using the D/S pairing.

\[ S_f = v + \frac{\kappa(S')}{D} m_0 + S \]  \hspace{1cm} (3.15)

The input \( v \) is chosen as in Eq. (3.14). As compared to the \( D/m_0 \) controller (3.10), an advantage of using \( S \) as the controlled output is that the resulting controllers (3.13) and (3.15) only require measurements of \( m_0 \) and \( S \).

The tuning parameters for the \( D/S \) and \( S_f/S \) controllers are chosen as before: \( \alpha_1 = 1 \text{ h}^{-1} \) and \( \alpha_0 = 0.25 \text{ h}^{-2} \). The closed-loop results for the same test as in Figure 3.6 are shown in Figures 3.7 and 3.8. Both controllers attenuate oscillations in the controlled output \( S \), but the damping is not as effective as for the \( D/m_0 \) controller.
controller due to higher sensitivity to sampling. Moreover the control moves are quite oscillatory and oscillations in the zeroth moment are barely attenuated. Consequently these two controllers are less desirable than the $D/m_0$ controller despite the additional measurement required to implement the $D/m_0$ controller.

The different closed-loop responses generated by the three feedback linearizing controllers are difficult to predict a priori. Of particular interest are the differences observed in the damping of the uncontrolled output. Below this behavior is analyzed for each controller using the closed-loop transfer function between the uncontrolled output and the setpoint.
Linearization of (3.2) and (3.7) at the steady-state operating point yields the
transfer function matrix:

\[
\begin{bmatrix}
m_0(s) \\
S(s)
\end{bmatrix} = \begin{bmatrix}
g_{11}(s) & g_{12}(s) \\
g_{21}(s) & g_{22}(s)
\end{bmatrix} \begin{bmatrix}
D(s) \\
D_f(s)
\end{bmatrix}
\]  
(3.16)

For the \(D/m_0\) controller denote the linearized controller transfer function as \(g_c(s)\).
The closed-loop system can be written as:

\[
m_0(s) = g_{11}(s)g_c(s)[m_0^*(s) - m_0(s)]
\]

\[
S(s) = g_{21}(s)g_c(s)[m_0^*(s) - m_0(s)]
\]

The control objective is to make \(m_0\) follow the second-order response defined by
(3.12): \(m_0(s) = g_d(s)m_0^*(s)\). The controller transfer function can be represented as:

\[
g_c(s) = \frac{1}{g_{11}(s)} \frac{g_d(s)}{1 - g_d(s)}
\]  
(3.17)

Therefore the closed-loop response of the uncontrolled output \(S\) has the form:

\[
S(s) = \frac{g_{21}(s)}{g_{11}(s)}g_d(s)m_0^*(s)
\]  
(3.18)

Similarly the closed-loop responses of the uncontrolled output \(m_0\) for the \(D/S\)
and \(S_f/S\) controllers are:
\[ m_0(s) = \frac{g_{11}(s)}{g_{21}(s)}g_d(s)S^*(s) \]
\[ m_0(s) = \frac{g_{12}(s)}{g_{22}(s)}g_d(s)S^*(s) \]

respectively. Since \( g_d(s) \) is chosen to have the form (3.12) and the open-loop transfer functions have the same poles, the zeros of the open-loop transfer functions \( g_{ij}(s) \) are primarily responsible for the observed differences in the closed-loop response of the uncontrolled output variables.

Figure 3.9 shows the zeros \( (z_i) \) of the transfer functions defined in (3.16). The transfer function \( g_{21} \) has several pairs of complex conjugate zeros very close to the imaginary axis, while the zeros of \( g_{11} \) are much further removed from the imaginary axis. This explains why oscillation in the substrate concentration are rapidly damped by the \( D/m_0 \) controller while the response of the zeroth moment is very oscillatory for the \( D/S \) controller. Similarly \( g_{22} \) has several pairs of complex conjugate zeros that are very close to the imaginary axis which causes the \( m_0 \) oscillations to be slowly damped. Also note that \( g_{12} \) has a RHP zero which would cause instability if the \( S_f/m_0 \) controller were designed and implemented.

Finally a MIMO feedback linearizing controller that regulates both \( m_0 \) and \( S \) by manipulation of \( D \) and \( S_f \) is designed. The design model consists of the zeroth moment equation (3.2), the substrate concentration equation (3.7) and the filtered substrate concentration equation (3.9). These equations are rewritten below in
Figure 3.9: Open-loop zeros of the zeroth moment-substrate concentration model.

The state-space form where $x = [m_0 \ S \ S']^T$, $u = [D \ DS_f]^T$ and $y = [m_0 \ S]^T$:

\[
\begin{align*}
\dot{x}_1 &= -x_1u_1 + r(x_3) \\
\dot{x}_2 &= u_2 - x_2u_1 - \frac{k(x_3)}{Y}m_0 \\
\dot{x}_3 &= \alpha(x_2 - x_3) \\
y &= \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix} x \tag{3.19}
\end{align*}
\]

Here $r(x_3) \equiv \int_0^\infty \Gamma(m, S')W(m, t)dm$ represents the growth rate of the cell number concentration; it is assumed to be known as before. Using standard input-output
decoupling [38] the following control law is obtained:

\[
\begin{bmatrix}
  u_1 \\
  u_2
\end{bmatrix} = \begin{bmatrix}
  -\frac{1}{y_1} & 0 \\
  -\frac{y_2}{y_1} & 1
\end{bmatrix} \begin{bmatrix}
  v - \begin{bmatrix}
    r(x_3) \\
    -k(x_3)y_1
  \end{bmatrix}
\end{bmatrix}
\] (3.20)

where \( v \) is the two-dimensional input vector of the feedback linearized system. Each input \( v_i \) is designed as in (3.11):

\[
v = \begin{bmatrix}
  \dot{m}_0^* \\
  \dot{S}^*
\end{bmatrix} - \begin{bmatrix}
  \alpha_1 & 0 \\
  0 & \beta_1
\end{bmatrix} y + \begin{bmatrix}
  \alpha_0 & 0 \\
  0 & \beta_0
\end{bmatrix} \int_0^t (y_{sp} - y) d\tau
\] (3.21)

where \( \alpha_i \) and \( \beta_i \) are tuning parameters and \( y_{sp} \) is the setpoint vector.

The closed-loop response (-) of the MIMO controller is shown in Figure 3.10. Also shown is the open-loop response (-----) corresponding to an oscillatory solution. The tuning parameters used are \( \alpha_1 = \beta_1 = 1 \text{ h}^{-1} \) and \( \alpha_0 = \beta_0 = 0.25 \text{ h}^{-2} \). As expected both output variables are effectively regulated to their setpoints when the controller is turned on at \( t = 4 \text{ hr} \). However, the control moves generated are excessive and preclude successful implementation of the controller on a real bioreactor. Therefore the \( D/m_0 \) feedback linearizing controller appears to be the best option for oscillation attenuation.

### 3.5 Summary and Conclusions

Bifurcation analysis have been performed on a population balance equation (PBE) model for budding yeast cultures grown in continuous bioreactors. The model ex-
hibits two supercritical Hopf bifurcations that are consistent with the spontaneous appearance and disappearance of sustained oscillations observed experimentally. However there are some discrepancies between experimental data and the model simulation results. These include the speed of transition to sustained oscillations in ramp tests and the perplexing question of possible existence of multiple stable solutions under the same operating conditions. It is believed that the first problem can be attributed to the lack of chemical structure in the PBE model. The second problem needs to be investigated with additional experimental work.
Four feedback linearizing controllers that employ different input-output variable pairings have developed and evaluated. Each controller is able to effectively attenuate oscillations in the controlled output variable(s), but the three SISO controllers have very different closed-loop responses for the uncontrolled output variable. The zeros of the linearized closed-loop system have been used to explain this behavior. Possible future work can be focused on: (i) experimental work aimed at determining the existence of multiple stable solutions; and (ii) dimensionality reduction of the PBE model to allow more detailed bifurcation analysis and the application of bifurcation-theoretic control techniques [17].
Chapter 4

Nonlinear Reduced Order Modeling of Yeast Cell Population Models

4.1 Introduction

Many investigators have shown that continuous cultures of the microorganism *Saccharomyces cerevisiae* (baker’s yeast) exhibit sustained oscillations in glucose limited environments under aerobic growth conditions [64, 68, 78, 80]. Recent research work has demonstrated that the oscillatory dynamics can be captured by unstructured cell population models in which each cell is distinguished according to its mass [59, 89]. The cell population model consists of a coupled set of nonlinear integro-partial differential equations. Discretization in the mass domain yields an approximate model with a large number of nonlinear ordinary differential equations. For a recent model by Mhaskar et al. [59], an accurate approximation consisting of 117 differential equations can be derived using orthogonal collocation on finite elements. Such high dimensional models are not well suited for nonlinear dynamic analysis and controller design. Furthermore, structured cell population models derived from flow cytometric measurements may be completely intractable due to their significantly increased complexity [76]. Consequently, there is considerable motivation to develop reduced-order models that capture the key features of cell population dynamics.
After fast initial transients the dynamics of many distributed parameter systems evolve in a much lower dimensional space than the order of an accurate discretized model. This suggests that a reduced order model can be derived by projecting the dynamics of the high-order discretized model onto an appropriate reduced dimensional subspace. The development of mathematically rigorous order reduction techniques for nonlinear partial differential equation models such as the yeast cell population model is an open problem. However, several semi-empirical order reduction methods including proper orthogonal decomposition (POD) [37] and approximate inertial manifolds [23, 40] can be used to construct a reduced dimensional space where the relevant dynamics evolve. In the POD approach, nonlinear model reduction is viewed as the problem of generating a convenient and in some sense optimal eigenfunction basis from which the reduced order model can be constructed. The basis functions of the reduced dimensional space are generated empirically by applying principal component analysis (PCA) to spatiotemporal data generated from open-loop simulation of the full-order discretized model. Galerkin projection of the full-order model onto the empirical eigenfunctions yields the reduced-order nonlinear model.

In this chapter, the POD-Galerkin method is used to a discretized model of yeast cell population dynamics to derive reduced-order nonlinear models that are more amenable to dynamic analysis and controller design. Several issues associated with the collection of a representative simulation data set that are critical for construc-
ition of a useful reduced-order model are illustrated. This requires a methodology for evaluating the accuracy of the reduced-order model with respect to the full-order discretized model. Dynamic simulation is used to assess the short-term accuracy of reduced-order models generated from different spatiotemporal data sets. While open-loop simulation is an invaluable tool for such comparisons, bifurcation analysis is shown to allow a more complete characterization of the reduced-order model dynamics. Zhang et al. has demonstrated that the yeast cell population model possesses a bifurcation structure where two critical values of the dilution rate separate regions of stable steady-state and stable periodic solutions [85]. The long term behavior of the reduced-order models is evaluated by comparing bifurcation diagrams of the full-order and reduced-order models. It is shown that a reasonably small number of empirical eigenfunctions is required to capture the dynamic behavior of the full-order model.

The remainder of the chapter is organized as follows. The yeast cell population model chosen for study is described in Section 4.2. The computational techniques used for nonlinear model reduction and bifurcation analysis are discussed in Section 4.3. The results of the model reduction study are presented and discussed in Section 4.4. A summary and conclusions are presented in Section 4.5.

### 4.2 Yeast Cell Population Model

The dynamic model chosen for investigation consists of a segregated description of the cell population and a structured description of the growth medium. The
resulting model is not a substitute for segregated yeast models that include a detailed description of the intracellular reactions. However, the structured medium description allows the model predictions to be compared with easily measured extracellular variables. Below the transient equations governing the cell population and the extracellular environment are presented. Additional details can be found in [59, 89].

Cell division in the budding yeast cell cycle is asymmetric. The smaller of the newborn cells obtained after division is referred to as a daughter cell while the larger cell is called a mother cell. Newborn daughter cells must grow to attain the size of a newborn mother cell (characterized here by the cell transition mass) before starting a budding cycle. By contrast, newborn mother cells bud shortly after being born. After budding has occurred, the bud grows while the mass of the mother cell remains essentially constant. The bud grows until the cell attains the size necessary for division (characterized by the cell division mass).

A population balance equation (PBE) that describes the evolution of the cell mass distribution is formulated as follows [22]:

\[
\frac{\partial W(m, t)}{\partial t} + \frac{\partial [K(S')W(m, t)]}{\partial m} = \int_0^\infty 2p(m, m')\Gamma(m', S')W(m', t)dm' - [D + \Gamma(m)]W(m, t)
\]

(4.1)

where: \(m\) is the cell mass; \(W(m, t)\) is the cell number density; \(K(S')\) is the overall single cell growth rate; \(S'\) is the effective substrate concentration (defined below);
$p(m, m')$ is the newborn cell probability function; $\Gamma(m, S')$ is the division intensity function; and $D$ is the dilution rate. The division intensity function is modeled as:

$$
\Gamma(m, S') = \begin{cases} 
0 & m \leq m_i^* + m_o \\
\gamma e^{-\epsilon(m-m_d^*)^2} & m \in [m_i^* + m_o, m_d^*] \\
\gamma & m \geq m_d^* 
\end{cases}
$$

(4.2)

where $m_i^*$ is the cell transition mass, $m_o$ is the additional mass that mother cells must gain before division is possible, $m_d^*$ is the cell division mass, and $\epsilon$ and $\gamma$ are constant parameters. The newborn cell probability function has the form:

$$
p(m, m') = A \exp[-\beta(m - m_i^*)^2] + A \exp[-\beta(m - m' + m_i^*)^2]
$$

(4.3)

when $m < m'$ and $m' > m_i^* + m_o$; the function is identically zero otherwise. Here $A$ and $\beta$ are constant parameters. This function yields two Gaussian peaks in the cell number distribution, one centered at $m_i^*$ corresponding to mother cells and one centered at $m_i^* - m'$ corresponding to daughter cells.

The functions (4.2) and (4.3) introduce dispersive effects into the PBE model that tend to counteract cell cycle synchrony [68] and dampen oscillatory dynamics. Sustained oscillations are obtained by modeling the dependence of the transition and division masses on the extracellular environment. The following saturation functions are used:
\[ m_i^*(S') = \begin{cases} 
  m_{i0} + K_i(S_t - S_h) & S' < S_t \\
  m_{i0} + K_i(S'_t - S_h) & S' \in [S_t, S_h] \\
  m_{i0} & S' > S_h 
\end{cases} \quad (4.4) \]

\[ m_d^*(S') = \begin{cases} 
  m_{d0} + K_d(S_t - S_h) & S' < S_t \\
  m_{d0} + K_d(S'_t - S_h) & S' \in [S_t, S_h] \\
  m_{d0} & S' > S_h 
\end{cases} \quad (4.5) \]

where: \( S_t, S_h, m_{i0}, m_{d0}, K_i \) and \( K_d \) are constants.

The structured medium model allows both glucose and ethanol to serve as substrates for cell growth. The following reaction sequence accounts for the three major metabolic pathways: glucose fermentation, glucose oxidation and ethanol oxidation:

\[ C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (4.6) \]

\[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad (4.7) \]

\[ C_2H_5OH + 3O_2 \rightarrow 2CO_2 + 3H_2O \quad (4.8) \]

The substrate balance equations are:

\[ \frac{dG}{dt} = D(G_f - G) - \int_0^\infty \left[ \frac{K_{gf}(G')}{Y_{gf}} + \frac{K_{go}(G')}{Y_{go}} \right] W(m, t) \, dm \quad (4.9) \]

\[ \frac{dE}{dt} = D(E_f - E) + \frac{92}{180} \int_0^\infty f(m) \frac{K_{gf}(G')}{Y_{gf}} W(m, t) \, dm - \int_0^\infty \frac{K_{eo}(E')}{Y_{eo}} W(m, t) \, dm \quad (4.10) \]
where: $G$ and $E$ are the glucose and ethanol concentrations, respectively; $G'$ and $E'$ are the effective glucose and ethanol concentrations, respectively; $G_f$ and $E_f$ are the feed glucose and ethanol concentrations, respectively; $Y_{gf}$, $Y_{go}$ and $Y_{eo}$ are constant yield coefficients; and the ratio $\frac{\alpha_2}{\alpha_0}$ represents the mass of ethanol produced per mass of glucose consumed in (4.6). The effective substrate concentrations model the lagged response of cell metabolism to changes in the extracellular environment:

\[
\frac{dG'}{dt} = \alpha_g(G - G') \tag{4.11}
\]
\[
\frac{dE'}{dt} = \alpha_e(E - E') \tag{4.12}
\]

where $\alpha_g$ and $\alpha_e$ are constants.

The glucose fermentation rate $K_{gf}$ is assumed to follow Monod kinetics with respect to glucose. The glucose oxidation rate $K_{go}$ and ethanol oxidation rate $K_{eo}$ are assumed to follow Monod kinetics with respect to both the substrate and the dissolved oxygen. Furthermore, the ethanol oxidation rate is assumed to be inhibited by glucose. The rate expressions are:

\[
K_{gf}(G') = \frac{\mu_{m_{gf}}G'}{K_{m_{gf}} + G'}
\]
\[
K_{go}(G') = \frac{\mu_{m_{go}}G'}{K_{m_{go}} + G'K_{m_{gd}} + O}
\]
\[
K_{eo}(E') = \frac{\mu_{m_{eo}}E'}{K_{m_{eo}} + E'K_{m_{ed}} + O K_{inhib}} \tag{4.13}
\]
where: \( O \) is the dissolved oxygen concentration; \( \mu_{mgf} \), \( \mu_{mgo} \) and \( \mu_{meo} \) are maximum consumption rates; \( K_{mgf}, K_{mgo}, K_{mgd}, K_{meo} \) and \( K_{med} \) are saturation constants; and \( K_{inhib} \) is a constant that characterizes the inhibitory effect of glucose on ethanol oxidation. The function \( f(m) \) in (4.10) is used to model production of ethanol by budded cells:

\[
f(m) = \begin{cases} 
0 & m \leq m^*_t \\
\gamma_e \exp \left[ -\epsilon_e (m - m^*_t - m_e)^2 \right] & m > m^*_t 
\end{cases} 
\]

(4.14)

where \( \gamma_e, \epsilon_e \) and \( m_e \) are constant parameters.

The liquid phase oxygen balance is written as:

\[
\frac{dO}{dt} = K_{lo}\alpha (O^* - O) - \int_0^\infty \left[ \frac{192}{180} \frac{Keo(G')}{Y_{go}} + \frac{96}{46} \frac{Keo(E')}{Y_{co}} \right] W(m,t)dm
\]

(4.15)

where: \( O^* \) is the saturation oxygen concentration; \( K_{lo} \) is the oxygen mass transfer coefficient; \( \alpha \) is the interfacial area per unit liquid volume; the ratios \( \frac{192}{180} \) and \( \frac{96}{46} \) account for differences in molecular weights of the reactants and products. The oxygen solubility is assumed to be governed by Henry’s law:

\[
O^* = H_O RT O_{out}
\]

(4.16)

where: \( H_O \) is the Henry’s rate constant for oxygen; \( O_{out} \) is oxygen partial pressure in the gas exhaust stream; \( T \) is the absolute temperature; and \( R \) is the gas constant.

The gas phase oxygen balance is:
\[
\frac{dV_gO_{out}}{dt} = F(O_{in} - O_{out}) - K_{lc}a(O^* - O)V_l
\]  \tag{4.17}

where: \(V_g\) and \(V_l\) are the gas phase and liquid phase volumes, respectively; \(F\) is the volumetric air feed flow rate; and \(O_{in}\) is the oxygen partial pressure in the air feed stream. The liquid phase carbon dioxide balance is:

\[
\frac{dC}{dt} = K_{lc}a(C^* - C) + \int_0^\infty \left[ \frac{264}{180} \frac{K_{go}(G')}{Y_{go}} + \frac{88}{46} \frac{K_{co}(E')}{Y_{co}} \right] W(m, t)dm \tag{4.18}
\]

\[
+ \int_0^\infty \left[ f(m) \frac{88}{180} \frac{K_{gf}(G')}{Y_{gf}} \right] W(m, t)dm
\]

where: \(C\) is the liquid phase carbon dioxide concentration; \(C^*\) is the saturation carbon dioxide concentration; \(K_{lc}\) is the carbon dioxide mass transfer coefficient; and the ratios \(\frac{264}{180}, \frac{88}{46}\) and \(\frac{88}{180}\) account for differences in molecular weights. The carbon dioxide solubility is modeled as:

\[
C^* = H_C(pH)RTC_{out}
\]  \tag{4.19}

where the carbon dioxide rate constant \(H_C\) is evaluated at a pH of 5.0; and \(C_{out}\) is the carbon dioxide partial pressure in the exhaust gas stream. The gas phase carbon dioxide balance is:

\[
\frac{dV_gC_{out}}{dt} = F(C_{in} - C_{out}) - K_{lc}a(C^* - C_{out})V_l
\]  \tag{4.20}
where \(C_{in}\) is the carbon dioxide partial pressure in the air feed stream. The model parameters are listed in Table 4.1 are obtained from [59].

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Variable</th>
<th>Value</th>
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</thead>
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<tr>
<td>(H_O)</td>
<td>0.0404 g/l/atm</td>
<td>(H_C)</td>
<td>1.48 g/l/atm</td>
</tr>
<tr>
<td>(V_g)</td>
<td>1 l</td>
<td>(V_i)</td>
<td>0.4 l</td>
</tr>
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<td>(K_{ic})</td>
<td>1500 h(^{-1})</td>
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<td>(F)</td>
<td>10 l/h</td>
</tr>
<tr>
<td>(T)</td>
<td>298 K</td>
<td>(O_{in})</td>
<td>0.21 atm</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
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<td>0.15 g/g</td>
<td>(\mu_{mgo})</td>
<td>(5 \times 10^{-11}) g/h</td>
</tr>
<tr>
<td>(Y_{co})</td>
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<td>(\mu_{mco})</td>
<td>(5 \times 10^{-11}) g/h</td>
</tr>
<tr>
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<td>(\epsilon)</td>
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<tr>
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<td>(\epsilon_c)</td>
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<tr>
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<td>(m_e)</td>
<td>(1 \times 10^{-11}) g</td>
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<td>(\beta)</td>
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<tr>
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<td>(m_{do})</td>
<td>(11 \times 10^{-11}) g</td>
</tr>
<tr>
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</tr>
<tr>
<td>(K_{mgd})</td>
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</tr>
<tr>
<td>(\alpha_g)</td>
<td>20</td>
<td>(\alpha_c)</td>
<td>20</td>
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### 4.3 Computational Techniques

#### 4.3.1 Numerical Model Solution

The cell population model presented in the previous section is comprised of a coupled set of nonlinear algebraic, ordinary differential and integro-partial differential equations. Numerical solution of the model is required to generate the spatiotemporal
data required for construction of the reduced-order nonlinear models. The standard approach is to spatially discretize the population balance equation (PBE) to obtain a finite number of nonlinear ordinary differential equations (ODEs) with time as the independent variable. A variety of numerical solution techniques based on finite difference, weighted residual and orthogonal collocation methods are available. An accurate approximation may require a large number of node points especially if there is more than a single dimension [73].

Zhu et al. [89] use orthogonal collocation on finite elements to discretize a simplified version of the yeast cell population model presented in Section 4.2. The cell mass domain is discretized into a number of finite elements, each of which contains several collocation points where the PBE is approximated by an ODE. Integral terms are approximated using Gaussian quadrature. The state vector of the resulting nonlinear ODE model consists of the cell number density at each collocation point, as well as the substrate and effective substrate concentrations. To obtain a sufficiently accurate discretization, 12 finite element and 8 internal collocation points on each finite element are employed. In this chapter, the same discretization scheme is utilized. The resulting model consists of 117 nonlinear ODEs.

4.3.2 Nonlinear Model Reduction

High dimensional models such as a discretized cell population model are not amenable to dynamic analysis and model-based controller design. Fortunately, most high dimensional models possess different time scales over which the dynamics evolve. The
model is viewed as having “fast modes” and “slow modes” according to the location of the associated eigenvalues in the complex space [43]. As the transient effect of the fast modes has a short duration, the relevant dynamic behavior of the model is determined mainly by the slow modes. If a high-order model possesses only a few slow modes, then it is effectively a low dimensional system. Using various model reduction techniques, the full-order model can be projected onto a much lower dimension subspace where the slow modes evolve. The resulting low-dimensional model should be a good approximation of the full-order model.

Because the discretized cell population model has a total of 117 nonlinear ODEs, it is too complex for effective analysis and control system design. However, 109 of the state variables represent the cell number distribution at different cell masses. As these state variables are highly correlated, it is envisaged that a significant dimensionality reduction is possible. As shown in Figure 4.1, the cell population model has a large number of fast modes. These observations motivate an attempt to perform dimensionality reduction on the cell population model to facilitate future dynamic analysis and controller design studies.

A large number of nonlinear model reduction techniques have been proposed in the literature. They can be categorized into three groups: (i) methods based on proper orthogonal decomposition (POD) [37]; (ii) methods based on approximate inertial manifolds (AIM) [7] and (iii) methods based on balanced truncation [48]. The POD method is used in this chapter since it has been shown to yield accurate
reduced-order models while accounting for the effect of input changes on the system behavior [73].

The POD method was originally proposed by Lorenz [52] and has been popularized by Lumley and others [37] for the study of dynamical features in complex fluid flows. More recently, POD has found wide application in solids and structures [37], image processing [74] and the design of controllers for PDE systems [73]. A brief outline of the POD method is presented below, a more detailed description can be found in [37]. Consider a nonlinear system of the general form
\[ \dot{x} = f[x(t), u(t)] \quad (4.21) \]

where \( x \in \mathcal{R}^n \) and \( u \in \mathcal{R}^m \) are the state and input vectors, respectively. The underlying idea of the POD method is to find an optimal low dimensional subspace of the state space in which the relevant dynamics of the original system evolve. The method requires extensive spatiotemporal data, either from the actual process or from simulation of an accurate model. For a given sampled data set \( \{x^{(1)}, \ldots, x^{(N)}\} \) of \( x(t) \), define \( R \) as the correlation matrix of the data

\[ R := \sum_{i=1}^{N} x^{(i)} x^{(i)*} \quad (4.22) \]

where \( x^{(i)} \) is the \( i \)-th snapshot of the state variables in the data set and \( x^{(i)*} \) is the complex conjugate of \( x^{(i)} \). Let \( \lambda_1 \geq \lambda_2 \geq \cdots \geq \lambda_n \) be the eigenvalues of \( R \), \( k \) be the rank of \( R \), and \( \phi_1, \phi_2, \ldots, \phi_k \) be orthogonal eigenvectors of \( R \) corresponding to the nonzero \( \lambda_i \). Each vector \( x^{(i)} \) can be written as

\[ x^{(i)} = \sum_{j=1}^{k} \alpha_{ij} \phi_j \]

where \( \alpha_{ij} = \langle x^{(i)}, \phi_j \rangle \), and \( \langle \phi_i, \phi_j \rangle = \delta_{ij} \). The optimal \( s \)-dimensional subspace approximation of the original state variables is given by

\[ \hat{x}^{(i)} = \sum_{j=1}^{s} \alpha_{ij} \phi_j \quad (4.23) \]
Denote $P := [\phi_1, \phi_2, \ldots, \phi_s]^T$ as the transformation matrix. The projection of $x$ on the subspace $S = \text{span}\{\phi_1, \phi_2, \ldots, \phi_s\}$ can be written as $y = Px$ where $y$ is a representation of $x$ in the new coordinates $\phi_i$. The approximation of $x$ is given by $\hat{x} = P^*Px \in S$ where $P^*$ is the complex conjugate of $P$. This subspace approximation is optimal in the sense that the “total energy” preserved

$$p = \frac{\sum_{i=1}^{s} \lambda_i}{\sum_{i=1}^{n} \lambda_i}$$

is maximized. The number of principal components $s$ retained in the reduced-order model should be chosen such that $p \geq 1$ to ensure good approximation of the full-order system.

Galerkin projection has been used extensively to construct reduced-order mathematical models of dynamical systems [37]. The basic idea is to project the full-order vector field on the tangent space of a $s$-dimensional subspace $S \subset \mathbb{R}^n$ of the original state space. Using the coordinates obtained from the POD methods, the resulting reduced order approximation is given by

$$\dot{\hat{y}}(t) = P f[P^*y(t), u(t)]$$

The POD-Galerkin method projects the dynamics onto the subspace containing most of the “energy” of the system. If all the eigenvectors corresponding to the nonzero eigenvalues of the correlation matrix $R$ are retained, then this subspace will
contain all the local dynamics. Clearly there is a trade-off between the extent of model reduction and the quality of the reduced-order model. The goal is to utilize as few basis functions as possible to ensure an “acceptable” approximation of the full-order dynamics.

In summary, the POD-Galerkin model reduction method involves:

1. Collection of a representative set of spatiotemporal process or model simulation data.

2. Extraction of an empirical eigenfunction basis from the data.

3. Construction of a reduced-order dynamical system by projection of the full-order vector field onto these basis functions.

The empirical nature of the method suggests that the first step is crucial for generating a useful approximate model. The data ensemble is the starting point for forming the reduced-dimensional subspace onto which the original state space is projected by the Galerkin procedure. All dynamics orthogonal to this subspace are neglected under the assumption that the resulting error will be “small”. In addition to the necessity for a large spatiotemporal data set, there are no a priori comprehensive guidelines for generation of a suitable ensemble from which the empirical basis functions will be extracted. A potentially representative ensemble can be obtained by combining spatiotemporal motions at several values of key operating parameters [19], mixing transients from different initial conditions distributed randomly around
relevant regions of the phase space [28] and collecting responses to perturbation of actuators from their nominal settings [6].

Because the primary interest of the reduced order modeling is to capture oscillatory dynamics of the cell population model, it is necessary to include transient data as well as stationary data. The ultimate goal is to develop a reduced-order model (ROM) suitable for model-based controller design. The ROM is required to provide reasonable predictions over a large operating regime. Consequently, it is necessary to incorporate simulation data for several parameter values that span the desired region of operation.

4.3.3 Bifurcation Analysis

Zhang and Henson [83] argued that bifurcation analysis allows more efficient and insightful analysis of bioreactor model behavior than is possible with dynamic simulation alone. The same argument applies to comparison of full-order model (FOM) and reduced-order model behavior. If a ROM has a bifurcation structure that closely matches that of the FOM, then the ROM is a good candidate for further analysis. It is important to note that bifurcation analysis only allows investigation of the long-term model dynamics. Therefore, dynamic simulation is necessary to analyze the short-term dynamic behavior.

In this chapter, only steady-state and periodic solutions of the yeast cell population model are studied. Steady-state solutions are located using the nonlinear equation solver NNES. Eigenvalues of the Jacobian matrix are computed at each
steady state to determine the local stability. A steady state where one or more eigenvalues cross the imaginary axis is known as a bifurcation point [46]. A continuation code based on the shooting method is used to locate periodic solutions and to determine their stability. The code requires a good initial guess of the state variables and the oscillation period at a particular operating condition. Such an initial point is readily obtained for a stable periodic solution by dynamic simulation. The ODE solver ODESSA is used for numerical integration. Limit cycles at different operating conditions can then be found via continuation. Stability of the periodic solutions is determined by examining the Floquet multipliers of the Poincaré map.

4.4 Results and Discussion

4.4.1 Full-Order Model

Mhaskar et al. [59] have demonstrated via dynamic simulation that the yeast cell population model can predict the coexistence of stable steady-state and stable periodic solutions at the same operating conditions as has been observed experimentally [81]. Figures 4.2 and 4.3 illustrate dynamic responses of the model for the inputs $D = 0.14 \, \text{h}^{-1}$ and $S_f = 30 \, \text{g/L}$ starting from two different initial conditions. An initial distribution that leads to a stable steady-state solution is shown in the top plot of Figure 4.2. As shown in the bottom plot, the initial distribution is sufficiently dispersed for the oscillations to slowly decay. Note that the final distribution shown in the top plot is very dispersed as compared to the initial distribution. A slightly
Figure 4.2: Transient response of the full-order model decaying to a steady-state solution [59].

less dispersed initial distribution that leads to sustained oscillations is shown in the top plot of Figure 4.3. In this case, the oscillation amplitude grows until a stable periodic solution is obtained. Two well-defined peaks that correspond to daughter and mother cell subpopulations are present in the final distribution. These tests confirm that the model is consistent with experimental observations that sustained oscillations are intimately related to cell cycle synchrony [77] and the formation of distinct cell subpopulations. Both attractors have significant regions of attraction, as will be verified by the bifurcation analysis results presented below. Given their
Figure 4.3: Transient response of the full-order model growing to a sustained oscillation [59].

similarity, the initial distributions shown in Figures 4.2 and 4.3 appear to be near the separatrix that divides the domains of attraction of the two solutions.

A bifurcation diagram for the full-order model is shown in Figure 4.4 where the dilution rate ($D$) is the bifurcation parameter and the glucose concentration ($G$) is chosen as a representative output variable. The model possesses a single stable steady-state solution (+) at low dilution rates. As the dilution rate is increased, a bifurcation (H1) occurs where the steady-state solution becomes unstable (o) and a stable periodic solution with oscillations of the amplitude indicated (−) appears. This Hopf bifurcation is accompanied by the appearance of large amplitude oscil-
Figure 4.4: Bifurcation diagram of the full-order model.

...lations. The stable periodic solution and the unstable steady-state solution coexist over a large range of dilution rates. As the dilution rate is increased further, a second bifurcation (H2) occurs where the periodic solution disappears and the steady-state solution regains its stability. This Hopf bifurcation is characterized by small amplitude oscillations. The Poincaré map indicates that the upper bifurcation is supercritical since all multipliers lie inside the unit circle, while the lower Hopf bifurcation is subcritical due to the presence of one Floquet multiplier outside the unit circle. It also discloses that the periodic solution branch undergoes a fold bifurcation (F) and changes its stability when one Floquet multiplier crosses the unit circle. Note
that there is a small range of dilution rates \( D \in (0.135 \text{ h}^{-1}, 0.145 \text{ h}^{-1}) \) near the subcritical bifurcation that supports both stable steady-state and periodic solutions. The domains of attraction of the two solutions are separated by an unstable periodic solution with oscillations of the amplitude indicated \((- - -\)) . This diagram provides a simple explanation for the dynamic simulation results presented above as the operating conditions are located in this range. It is important to note that a earlier version of the model without the structured medium description by Zhu et al. [89] does not exhibit a subcritical Hopf bifurcation. Therefore, the current model represents a significant improvement because it more faithfully reproduces experimentally observed behavior.

Before performing POD-Galerkin model reduction, it is useful to investigate the spectrum of the FOM to determine if such a reduction is expected to be beneficial, i.e., if the discretized model has a large number of fast modes and small number of slow modes. Figure 4.1 shows the spectrum of the FOM at an unstable steady state solution where \( D = 0.17 \text{ h}^{-1} \) and \( S_f = 30 \text{ g/L} \). The model has a total of 117 eigenvalues, many of which have very large negative real parts. For clarity, only the 64 eigenvalues which have real parts greater than \(-30\) are shown in the figure. As shown in the inset, there are also approximately twenty slow modes corresponding to eigenvalues which have real parts greater than \(-0.5\). Investigation of the model spectrum at different operating conditions yields similar results. This suggests that the ROMs derived will require on the order of twenty basis functions
to produce accurate predictions. Also note that the arrangement of eigenvalues suggests the possible existence of continuous and discrete spectrum for this problem. The Hopf bifurcation is clearly associated with discrete spectrum crossing and results in coherent oscillations of the population. The observation of apparently continuous spectrum relatively close to the imaginary axis is worth further exploration since it may have implications for the separation of time scales in the system.

4.4.2 Reduced-Order Model: Single Data Set

Before presenting the ROM results, a few practical issues involved in applying the POD-Galerkin method are discussed. First, it is important to scale the FOM state variables such that they have comparable magnitude. Since the POD method is concerned only with the “total energy” in the data ensemble, important variables with small magnitude may not be adequately reflected in the ROM in the absence of scaling. In fact, it failed to generate useful ROMs with unscaled raw data. Second, the scaled raw data are used directly for ROM construction. Some researchers [19, 48] have suggested that the ensemble mean should be subtracted from the data before model reduction. For a single data set, it is found that this approach provides no discernible advantage. Furthermore, the ensemble mean does not have a physical meaning when multiple data sets at different parameter values are combined to generate a “global” reduced-order model. Therefore, the mean value is not subtracted from the data ensemble.
Figure 4.5: Performance of ROMs based on a single data set at $D = 0.17 \text{ h}^{-1}$.

To study the effectiveness of POD-Galerkin model reduction, a ROM derived from a single set of transient simulation data at a fixed operating condition ($D = 0.17 \text{ h}^{-1}$ and $S_I = 30 \text{ g/L}$) is first studied. The training data set consists of 600 snapshots of the 117 state variables during the first 60 hours of the open-loop simulation shown in the first subplot of Figure 4.5. This data set contains information on both the transient phase and the fully developed oscillations. A ROM constructed from this data set is expected to capture both the short-term and long-term dynamics of the FOM. A variety of ROMs with different numbers of principal components (PCs) were constructed and compared to the FOM. The results are summarized below:
• $\leq 6$ PCs: integrator fails to converge.

• 7–8 PCs: the transient response exhibits large errors, while the sustained oscillation have an amplitude comparable to that of the full-order model. The mean value of the fully developed oscillations has significant offset.

• 9–16 PCs: integrator fails to converge.

• 18–20 PCs: the ROMs yields reasonably accurate predictions.

• 25+ PCs: the ROMs yields almost perfect predictions.

The need to maintain approximately 20 PCs to obtain an accurate ROM is not particularly desirable, but it is consistent with the earlier analysis of the model spectrum. The result that ROMs with 7 or 8 PCs can yield reasonable results while ROMs with 9–16 PCs fail is surprising. Although not studied here, additional order reduction may be possible with more advanced techniques such as nonlinear Galerkin projection [5].

Figure 4.5 provides a comparison of two ROMs with 20 and 25 PCs with the FOM. The original 117 state variables are reconstructed from the ROM simulation results, and the reconstructed glucose concentration is plotted in the figure. The 25-PC ROM provides a very good match to the FOM, both in terms of short-term transients and long-term sustained oscillations. The 20-PC ROM not only inaccurately predicts the short-term dynamics, but it also yields sustained oscillations with an incorrect period and a significant offset in mean glucose concentration as
Figure 4.6: Transient response of the 25-PC ROM for $D = 0.18 \text{ h}^{-1}$.

compared to the FOM. Phase portraits corresponding to sustained oscillations of the three models are shown in the lower subplot of Figure 4.5 where the glucose concentration ($G$) and the gas phase $CO_2$ concentration ($C_{\text{out}}$) are selected as representative variables. The 20-PC ROM exhibits large errors, while the trajectory for the 25-PC ROM is almost identical to that of the FOM. Extensive simulation tests confirm that ROMs based on 25+ PCs also provide very accurate predictions.

A validation test is performed to evaluate the ability of the 25-PC ROM to predict dynamic behavior under different operating conditions. Figure 4.6 shows an open-loop simulation for $D = 0.18 \text{ h}^{-1}$ and $S_f = 30 \text{ g/L}$. The ROM does not
effectively capture the dynamic behavior of the FOM at this operating condition.
Because the ROM ultimately will be used for model-based controller design, it is clear that a single data set at a fixed dilution rate will not be satisfactory. Additional FOM data representing the operating regime of interest must be collected for the ROM to yield accurate predictions over a meaningful range of dilution rates.

4.4.3 Reduced-Order Model: Oscillatory Range

Figure 4.7 shows a training data set consisting of five sets of transient data obtained from open-loop simulations of the FOM under different operating conditions that
support sustained oscillations. The data sets are generated by fixing $S_f$ at 30 g/L and setting $D$ at five different values: 0.15, 0.16, 0.17, 0.18, and 0.19 h$^{-1}$. Each data set consists of 400 snapshots of the FOM state variables at different phases of the simulation where the oscillations have very small amplitude (100 points), the oscillations are somewhat developed (100 points), the oscillations are almost fully developed (100 points) and the oscillations are fully developed (100 points). Data set are constructed in this manner to avoid large data sets that would result from direct sampling of an oscillatory simulation. By including transient responses and sustained oscillations at different operating conditions, the derived ROMs are expected to capture the short-term and long-term dynamics more “globally” than is possible with a single data set.

Figure 4.8 provides a comparison of two ROMs derived from the data set in Figure 4.7 and the FOM for two dilution rates ($D = 0.15$ h$^{-1}$ and $D = 0.18$ h$^{-1}$) contained within the training data set. The 40-PC ROM produces an almost perfect match of the glucose concentration. The 30-PC ROM provides satisfactory approximation for $D = 0.18$ h$^{-1}$, but it is unstable for $D = 0.15$ h$^{-1}$. Extensive simulation studies verify that ROMs with 40+ PCs also yield highly accurate approximation. Figures 4.9 and 4.10 compare the ROM and FOM responses for two operating conditions outside the range of the training data set ($D = 0.20$ h$^{-1}$ and $D = 0.14$ h$^{-1}$). The 40-PC ROM provides reasonably accurate extrapolation, while the 30-PC ROM is not able to accurately reproduce the FOM dynamics.
Bifurcation analysis allows a more detailed study of the long-term dynamics of the ROMs. Figure 4.11 shows a comparison of the one-parameter bifurcation diagrams of the 35-PC and 40-PC ROMs and the FOM. Within the oscillatory range, the unstable steady-state solutions of the 40-PC ROM are quite close to those of the FOM. The predicted steady states outside the oscillatory region are less accurate due to a lack of training data. The amplitudes of the 40-PC ROM limit cycles match those of FOM quite accurately over a large range of dilution rates. Furthermore, the 40-PC ROM correctly predicts the existence of the two Hopf bifurcations with the lower bifurcation being subcritical and the upper bifurcation being supercritical.
Figure 4.9: Performance of ROMs outside the training range for $D = 0.20$ h$^{-1}$.

The locations of the two bifurcation points are very close to those of the FOM. On the other hand, the 40-PC ROM predicts a significantly larger operating space where stable steady-state and stable periodic solutions coexist. These results demonstrate that the 40-PC ROM represents a good approximation of the FOM despite lack of training data outside the oscillatory region. To achieve better agreement at lower dilution rates within the oscillatory region and for dilution rates outside the oscillatory region, it is necessary to utilize a more complete training data set. The predictions of the 35-PC ROM are far less satisfactory. In particular, the 35-PC ROM does not capture the lower Hopf bifurcation or the existence of multiple stable
Figure 4.10: Performance of ROMs outside the training range for $D = 0.14$ h$^{-1}$.

solutions. Consequently, this model is useful only in the upper range of oscillatory solutions.

### 4.4.4 Reduced-Order Model: Global Behavior

Figure 4.12 shows a training data set used to obtain better predictions of the global FOM behavior. A total of six distinct data sets, each with 400 snapshots, are utilized. The data sets are collected at the same feed substrate concentration $S_f = 30$ g/L and six different dilution rates $D$ of $0.13$ h$^{-1}$, $0.14$ h$^{-1}$, $0.16$ h$^{-1}$, $0.18$ h$^{-1}$, $0.20$ h$^{-1}$ and $0.21$ h$^{-1}$ from left to right in the figure. For dilution rates $D = 0.13$ h$^{-1}$ and $D = 0.21$ h$^{-1}$ where the FOM has only one stable steady-state solution, each
Figure 4.11: Bifurcation diagram of ROMs constructed from multiple data sets within the oscillatory range.

data set consists of 300 snapshots of oscillations decaying to the steady state and 100 snapshots of the steady-state solution itself. For $D = 0.14 \, h^{-1}$ where multiple stable solutions are supported, the data ensemble contains 200 snapshots of growing oscillations and sustained oscillations and 200 snapshots of decaying oscillations and the steady-state solution. For the other three dilution rates where only sustained oscillations exist, the data ensemble is constructed as in Figure 4.7.

Figure 4.13 provides a comparison of the transient responses of 35-PC and 40-PC ROMs and the FOM at $D = 0.18 \, h^{-1}$. With 40 basis functions retained, the ROM predictions are very close to those of the FOM. By contrast, the ROM with
35 principal components is not able to approximate the plant dynamics. Figure 4.14 shows a comparison of the 40-PC ROM and the FOM at \( D = 0.14 \) h\(^{-1} \) for the set of initial conditions shown in the Figures 4.2 and 4.3. The FOM initial conditions are mapped into the reduced-dimensional space using the transformation matrix \( P \) to generate the initial conditions for the ROM. The ROM captures the convergence to the two different solutions, although the transient responses exhibit some small errors. Although not shown in the figure, it is worth mentioning that open-loop simulations with the 35-PC ROM lead to integration failure.
Figure 4.13: Performance of the global ROMs for $D = 0.18 \text{ h}^{-1}$.

Figure 4.15 shows the bifurcation diagrams for 40-PC and 50-PC ROMs and the FOM. The 40-PC ROM provides very close agreement near the lower bifurcation point, including the predicted range of multiple stable solutions and the predicted steady-state solutions outside the oscillatory range. This is attributable to utilizing a more global data set for ROM construction. This improved predictive capability is accompanied by less accurate predictions near the upper bifurcation point. In comparison, the 50-PC ROM is slightly better in predicting the oscillation bounds but less accurate in locating the lower Hopf bifurcation point and the fold bifurcation
point of the periodic solutions. Many other tests of ROMs with different principal components have been performed. The ROMs with higher number of basis functions showed little improvement in the approximating the overall bifurcation diagram over the 40-PC ROM. Taking the computational cost into consideration, the 40-PC ROM represents a good approximation of the FOM.

One may easily notice the differences between the 40-PC ROM built from the oscillatory data set (Figure 4.11) and the 40-PC ROM built from the global data set (Figure 4.15). The former model provides more accurate prediction of the upper and lower oscillation limits over a wide operating range, while the latter model is
Figure 4.15: Bifurcation diagram of ROMs constructed from global data set.

superior in capturing the locations of the two bifurcation points. These results are attributable to differences between the training data sets since the approximation capability is highly dependent on the data used. As the relative weighting of oscillatory data in the global data set is significantly reduced with the introduction of data outside the oscillatory range, the prediction accuracy of the oscillation amplitudes is expected to be sacrificed. Clearly, a different data set could be constructed to obtain a ROM which provides better predictions in the oscillatory range at the expense of less accurate predictions of the bifurcations point locations.
These results again emphasize that the POD method is empirical and data-driven. The training data sets used in this study are admittedly heuristic, albeit they are chosen according to some reasonable guidelines. Therefore, the ROM obtained can not be considered as optimal in a practical sense. Furthermore, the choice of satisfactory ROMs is based largely on individual judgment. General guidelines include, but are not limited to short-term versus long-term prediction capabilities, dimension versus model accuracy and model accuracy versus model robustness.

4.5 Summary and Conclusions

Model order reduction of a discretized yeast cell population balance model has been studied using a combination of proper orthogonal decomposition (POD) and Galerkin projection. The collection of a representative spatiotemporal data set from which the basis functions of the reduced-order model (ROM) are constructed was shown to be critical. Dynamic simulation and bifurcation analysis results demonstrate that accurate ROMs can be generated with roughly one-third of the differential equations of the full-order model. The ROMs yield very good short-term and long-term predictions over a wide range of operating conditions. Despite the significant dimensionality reduction, accurate ROMs are composed of approximately 40 nonlinear differential equations. Consequently, additional order reduction methods such as nonlinear Galerkin projection [5] are currently being pursued. In particular, I plan to explore the interplay of low-dimensionality and the continuous spectrum
suggested by Figure 4.1. Additional future work will focus on the use of ROMs for model-based control of continuous yeast bioreactors.
Chapter 5
Conclusions and Recommendations

Bioreactors have very complex dynamic behaviors. Understanding and appropriate modeling of these behaviors will provide great potential for the ultimate purpose of controller design to enhance product quality and process operability. This thesis has focused on the dynamic analysis, reduced order modeling and nonlinear control of such bioreactors. In this chapter, the most important results are summarized and suggestion for future research directions are outlined.

5.1 Dynamic Analysis Techniques

In Chapter 2, the capability of bifurcation analysis in studying the complex dynamic behaviors of nonlinear systems is illustrated. Three continuous bioreactor models that exhibit complex steady-state and transient behavior are studied. Bifurcation analysis is shown to be a much better dynamic analysis tool than open-loop simulation: (i) it is much more efficient since it avoids the time consuming integration, especially when the transient phase of the system is extremely slow; (ii) it studies the “complete” model dynamics while simulation can only have a limited number of tests; (iii) it can disclose hidden model behaviors that are unable to be tracked by simulation; and (iv) it provides straightforward mathematical explanations to complex model behaviors. Important features of the three models studied that were not
observed by the original authors are revealed through bifurcation analysis. These features include lack of model robustness to small parameter variations, apparent inconsistencies between model structure and experimental data, and the existence of multiple modes under the same operating conditions. These case studies suggest that bifurcation analysis is a very powerful tool for analyzing low-dimensional bioreactor models and should be used for model validation whenever possible. The study of model behavior versus parameter also provides a very effective approach for determining unknown model parameters. Bifurcation analysis is also used to study population balance models for yeast cultures in Chapter 3 and 4. It is shown to be very effective in comparing and discriminating between several candidate models.

5.2 Model Order Reduction

In Chapter 4, the major effort is to seek a model order reduction of a discretized segregated and unstructured yeast cell population balance model. Proper orthogonal decomposition is used in conjunction with linear Galerkin projection for this purpose. Several practical issues are discussed concerning the application of this method. The collection of a representative spatiotemporal data set from which the basis functions of the reduced-order model (ROM) are constructed was shown to be most critical. A various set of reduced-order models obtained from different data sets and with different number of basis functions are tested and compared to the full-order model via dynamic simulations and bifurcation analysis. Results demonstrate that accurate ROMs can be generated with roughly one-third of the
differential equations of the full-order model (FOM). The candidate ROMs provide very good short-term and long-term approximations to the FOM over a wide range of operating conditions.

It should be mentioned that the ultimate objective of seeking model order reduction is to use the ROMs for model-based control of continuous yeast bioreactors. Although significant dimensionality reduction has been achieved, accurate ROMs still contains of around 40 nonlinear differential equations which is not very desirable for controller design purpose. Simple investigation of the spectrum of the Jacobian matrix of the FOM at a representative operating condition reveals that the inherent model structure makes significant further dimension reduction impossible. Noticing the possible existence of discrete and continuous spectrum for the original population balance model [84], further investigation of the model spectrum is recommended to be performed.

Further research in seek of lower-dimension usable ROMs could follow one of the following two ways: (i) based on the controller design requirements, construct a set of ROMs which have good predictions in a small operating range; (ii) use of the so-called nonlinear Galerkin projection procedure [5] instead of the “flat” Galerkin method employed in this study. The first method is very simple to implement and is a mechanism commonly used by industries. Smaller operating range requires less training data and conceivably less basis function. The nonlinear Galerkin method is similar to the singular perturbation method [43], the states in the reduced-order
model are rearranged in the order of time scales, the ordinary differential equations (ODE) for the fast modes can be approximated by algebraic equations. The resulting model is then a set of differential algebraic equations (DAE). There are a variety of ways to implement this approximation and the training data might have to be different from the linear POD-Galerkin method [5].

In addition, other model reduction techniques such as AIM and balanced truncation methods can be further tested and compared with the POD method.

5.3 Nonlinear Control

Besides the bifurcation analysis study for model validation and discrimination of a yeast cell population balance model. The major focus of Chapter 3 has been on developing of a simple and effective feedback linearization controller. The controller design is based on a simplified model which consists of a zeroth moment equation derived from the PBE model and the substrate concentration balance equations. Four controllers that employ different input-output variable pairings have developed and evaluated. Each controller is able to effectively attenuate oscillations in the controlled output variable(s), but the three SISO controllers have very different closed-loop responses for the uncontrolled output variable. The zeros of the linearized closed-loop system have been used to explain this behavior. It is worth mentioning that the feedback linearization control method is based on the assumption that the new born cell rate can be measured adequately fast which is not reasonable as of
the current measuring technology. However, emerging new technologies such as flow
cytometric [75] make it promising.

Major research effort can be focused on the use of model based control techniques
such as linear or nonlinear model predictive control (LMPC, NMPC) in combina-
tion with accurate reduced order models. Studies of applying LMPC on ROMs with
dimension up to 40 has been positive. However, it does not offer much improve-
ment as the work by Zhu et al. [89]. Preliminary studies has disclosed that the
major challenge for the successful application of an NMPC controller is the heavy
computation load caused by the nonlinear programming (NLP) solver. Studies em-
ploying the simultaneous simulation and model solving approach [58] requires the
discretization of the ODEs in time to generate a set of algebraic equations (AE).
Using a ROM of order 40, after this discretization, the resulting model consists of
thousands of AEs which is a formidable task for most NLP solvers. Using the widely
known NLP solver SNOPT, a normal NMPC computation step for sampling period
of 1 hr can take a Pentium III/866MHz personal computer about an hour of CPU
time. Furthermore, the NLP solver often fails to converge or simply finds a local
minima. Hence, the overall implementation of NMPC control has not been success-
ful. Use of ROMs with less basis functions has not turn out positive results either.
Future study should first focus on further model order reduction as mentioned in
the previous section. It is also advisable to employ less accurate NMPC approaches
such as hte successive linearization method [32]. In this method, the ODE model is
linearizes at each iteration and an LMPC control input is computed at each step. This method avoids accurate discretization of the ODE model in time and thus can greatly reduce the computational cost.
Bibliography


control of continuous yeast bioreactors using cell population models. *Chem.
Appendix A

Supplement to Chapter 3

A.1 Discretized Population Balance Model

\[
\begin{align*}
\dot{x}_i &= \frac{1}{h (K_m + S') \sum_j A_{ij}x_j + 2h \sum_j PW_{ij} \Gamma_j x_j} - (D + \Gamma_i)x_i \\
\dot{S} &= \frac{1}{h (K_m + S') \sum_i w_i x_i} \\
\dot{S}' &= \alpha(S - S') \\
\end{align*}
\]  

(A.1)

A.2 Analytical Jacobian Matrix of the PBE model

Denote the Jacobian matrix of the above model as

\[
J = \left[ \frac{\partial f_i}{\partial x_j} \right] \quad i, j = 1, n + 2
\]

the elements in J are:

\[
\begin{align*}
\frac{\partial f_i}{\partial x_j} &= -\frac{1}{h (K_m + S') \sum_j A_{ij}x_j + 2h \sum_j PW_{ij} \Gamma_j x_j} - (D + \Gamma_i) \delta_{ij} \\
\frac{\partial f_i}{\partial x_{n+1}} &= 0 \\
\frac{\partial f_i}{\partial x_{n+2}} &= -\frac{1}{h (K_m + S')^2 \sum_j A_{ij}x_j + 2h \sum_j \frac{\partial PW_{ij} \Gamma_j x_j}{\partial x_{n+2}}} - x_i \frac{\partial \Gamma_i}{\partial x_{n+2}}
\end{align*}
\]
\[
\frac{\partial f_{n+1}}{\partial x_i} = -\frac{h}{Y} \frac{\mu_m S_l}{K_m + S_l} w_i
\]
\[
\frac{\partial f_{n+1}}{\partial x_{n+1}} = -D
\]
\[
\frac{\partial f_{n+1}}{\partial x_{n+2}} = \frac{h}{Y} \frac{\mu_m K_m}{(K_m + S_l)^2} \sum_i w_i x_i
\]
\[
\frac{\partial f_{n+2}}{\partial x_i} = 0
\]
\[
\frac{\partial f_{n+2}}{\partial x_{n+1,2}} = [\alpha, -\alpha]
\]

where \(i, j = 1, n\), and the terms:

\[
\frac{\partial \Gamma_i}{\partial x_{n+2}} = \frac{\partial \Gamma_i}{\partial m_d} \frac{\partial m_d^*}{\partial x_{n+2}} = \begin{cases} -2\epsilon(m_d^* - m_i)\Gamma_i K_d & \text{for } S_l \in [S_t, S_h) \text{ and } m_i \in (m_d^* + m_o, m_d^*) \\ 0 & \text{otherwise} \end{cases}
\]

\[
\frac{\partial PW_{ij} \Gamma_j x_j}{\partial x_{n+2}} = \Gamma_j x_j \frac{\partial PW_{ij}}{\partial x_{n+2}} + PW_{ij} x_j \frac{\partial \Gamma_j}{\partial x_{n+2}}
\]

\[
\frac{\partial PW_{ij}}{\partial x_{n+2}} = \nu w_j \frac{\partial P_{ij}}{\partial x_{n+2}} = \nu w_j \frac{\partial P_{ij}}{\partial m_i^*} \frac{\partial m_i^*}{\partial x_{n+2}}
\]

\[
\frac{\partial P_{ij}}{\partial m_i^*} = -2A\beta((m_i^* - m_i)\exp\{\cdots\} + (m_i^* + m_i - m_j)\exp\{\cdots\})
\]

where

\[
\frac{\partial m_i^*}{\partial x_{n+2}} = \begin{cases} K_t & \text{for } S_l \in [S_t, S_h) \\ 0 & \text{otherwise} \end{cases}
\]
# Appendix B

## Supplement to Chapter 4

### B.1 Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C )</td>
<td>Dissolved carbon dioxide concentration ([g/l])</td>
</tr>
<tr>
<td>( C^* )</td>
<td>Saturation carbon dioxide concentration ([g/l])</td>
</tr>
<tr>
<td>( C_{\text{in}} )</td>
<td>Carbon dioxide partial pressure in air feed stream ([\text{atm}])</td>
</tr>
<tr>
<td>( C_{\text{out}} )</td>
<td>Carbon dioxide partial pressure in gas exhaust stream ([\text{atm}])</td>
</tr>
<tr>
<td>( D )</td>
<td>Dilution rate ([\text{hr}^{-1}])</td>
</tr>
<tr>
<td>( E )</td>
<td>Ethanol concentration ([g/l])</td>
</tr>
<tr>
<td>( E' )</td>
<td>Filtered ethanol concentration ([g/l])</td>
</tr>
<tr>
<td>( E_f )</td>
<td>Ethanol feed concentration ([g/l])</td>
</tr>
<tr>
<td>( F )</td>
<td>Volumetric flow rate of air ([\text{l/h}])</td>
</tr>
<tr>
<td>( G )</td>
<td>Glucose concentration ([g/l])</td>
</tr>
<tr>
<td>( G' )</td>
<td>Filtered glucose concentration ([g/l])</td>
</tr>
<tr>
<td>( G_f )</td>
<td>Glucose feed concentration ([g/l])</td>
</tr>
<tr>
<td>( H_o )</td>
<td>Henry's law constant for oxygen ([g/l/\text{atm}])</td>
</tr>
<tr>
<td>( H_c )</td>
<td>Henry’s law constant for carbon dioxide ([g/l/\text{atm}])</td>
</tr>
<tr>
<td>( K_d )</td>
<td>Slope of division mass in saturation function ([l])</td>
</tr>
</tbody>
</table>
\( K_{eo} \) Ethanol oxidation rate \([\text{g/hr}]\)

\( K_{gf} \) Glucose fermentation rate \([\text{g/hr}]\)

\( K_{go} \) Glucose oxidation rate \([\text{g/hr}]\)

\( K_{inhib} \) Inhibition constant for ethanol oxidation \([\text{g/l}]\)

\( K_{lc} \) Carbon dioxide mass transfer coefficient \([\text{m/hr}]\)

\( K_{lo} \) Oxygen mass transfer coefficient \([\text{m/hr}]\)

\( K_{meo} \) Ethanol saturation constant for ethanol oxidation \([\text{g/l}]\)

\( K_{mgf} \) Glucose saturation constant for glucose fermentation \([\text{g/l}]\)

\( K_{mgo} \) Glucose saturation constant for glucose oxidation \([\text{g/l}]\)

\( K \) Overall single cell growth rate \([\text{g/hr}]\)

\( K_t \) Slope of transition mass in saturation function \([\text{l}]\)

\( O \) Dissolved oxygen concentrations \([\text{g/l}]\)

\( O^* \) Saturation dissolved oxygen concentration \([\text{g/l}]\)

\( O_{out} \) Oxygen partial pressure in gas exhaust stream \([\text{atm}]\)

\( O_{in} \) Oxygen partial pressure in the feed stream \([\text{atm}]\)

\( R \) Gas constant \([\text{l atm/mole/K}]\)

\( S_l, S_h \) Substrate concentration limits in the saturation functions \([\text{g/l}]\)

\( S' \) Filtered substrate concentration \([\text{g/l}]\)

\( T \) Absolute temperature \([\text{K}]\)

\( V_g \) Reactor gas phase volume \([\text{l}]\)

\( V_l \) Reactor liquid phase volume \([\text{l}]\)
$W$ Distribution of states $[\#/g]$

$\dot{x}$ Model state vector

$Y_{eo}$ Yield coefficient for ethanol oxidation $[g/g]$

$Y_{gf}$ Yield coefficient for glucose fermentation $[g/g]$

$Y_{go}$ Yield coefficient for glucose oxidation $[g/g]$

$a$ Interfacial area per unit liquid volume $[m^{-1}]$

$f$ Ethanol production function

$f^d$ Objective function for parameter estimation from dynamic data

$f^s$ Objective function for parameter estimation from steady-state data

$k_{go}$ Dissolved oxygen saturation constant for glucose oxidation $[g/l]$

$k_{eo}$ Dissolved oxygen saturation constant for ethanol oxidation $[g/l]$

$m_c$ Mass cells must attain to produce ethanol $[g]$

$m^*_d$ Cell division mass $[g]$

$m^*_t$ Cell transition mass $[g]$

$m_o$ Mass above $m^*_t$ a mother cell must gain before division is possible $[g]$

$m_{do}$ Saturation value for transition mass $[g]$

$m_{to}$ Saturation value for division mass $[g]$

$p$ Newborn cell mass distribution function

$u$ Input vector

$y$ Measured variables in plant

$\hat{y}$ Measured variables as predicted by model
\( \alpha_c \) Filter constant for ethanol [hr\(^{-1}\)]

\( \alpha_g \) Filter constant for glucose [hr\(^{-1}\)]

\( \Gamma \) Division intensity function

\( \gamma \) Pre-exponential factor in division intensity function

\( \gamma_c \) Pre exponential factor in ethanol production function

\( \epsilon \) Division intensity function slope

\( \epsilon_e \) Ethanol production function slope

\( \theta_1 \) Unknown parameter values that are estimated

\( \theta_2 \) Unknown parameter values that are not estimated

\( \theta_{fix} \) Parameter values derived from literature

\( \theta_{est} \) Parameter values available for estimation

\( \lambda \) Eigenvalues of the principle component matrix

\( \mu_{meo} \) Maximum ethanol oxidation rate [g/hr]

\( \mu_{mgo} \) Maximum glucose oxidation rate [g/hr]

\( \mu_{mgf} \) Maximum glucose fermentation rate [g/hr]
Vita

Yongchun Zhang was born in Tongxiang, Zhejiang Province, China, on February 20, 1974. He attended elementary and junior high schools in his home town. He then attended Jiaxing No. 1 High School. In 1991, due to his academic excellence, he was admitted to the Mixed Class, the top-three-percent-student honors program in Zhejiang University, with the college entrance exam waived. He received his bachelor of science degree in chemical engineering in June 1995. He then worked as a systems engineer in the Sino-Stride Electronic Co. Ltd., for two years. In August 1997, he began his doctoral studies in the Chemical Engineering Department at Louisiana State University under the supervision of Prof. Michael A. Henson. After Prof. Henson’s resignation from L.S.U. in early 2002, he continued his work under the direction of Prof. Martin A. Hjortsø and received the degree of Doctor of Philosophy in chemical engineering in August 2002.