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Dynamic modeling of microbial cell populations

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Microbial cultures are comprised of heterogeneous cells that differ according to their size and intracellular concentrations of DNA, proteins and other constituents. Recent advances have been made in cell population modeling, which allow the effects of cell heterogeneity on culture dynamics and metabolite production to be predicted. If the intracellular state can be captured with a few variables, the population balance equation framework is a viable modeling approach. The cell ensemble modeling technique is better suited for the development of population models that include more detailed descriptions of cellular metabolism and/or cell-cycle progression.

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Abbreviation
PBE population balance equation

Introduction

By viewing a microbial culture as a homogeneous mixture of identical cells, experimental results and mathematical models representative of average cell behavior are readily scaled to the cell population level. However, individual cells exhibit heterogeneity as a result of small differences in their cellular metabolism and cell-cycle dynamics. Repeated movement through the cell cycle yields a heterogeneous population in which individual cells differ according to their size and intracellular state. Unless a synchronous population is established by exploiting natural mechanisms [1] or through artificial means [2], the average cell behavior is not representative of the entire population. This motivates the development of experimental and modeling techniques that account for heterogeneities present at the single-cell level.

Cell heterogeneities can have a significant impact on microbial culture dynamics and the production of key metabolites. Several examples are given below.

Yeasts can exhibit glycolytic oscillations at the single-cell level owing to the autocatalytic activity of the enzyme phosphofructokinase. In the absence of a synchronization mechanism, random variations in energy metabolism would cause individual cells to oscillate out of phase. Instead, secreted acetaldehyde causes dynamic synchronization of the individual cells and results in sustained oscillations at the cell population level [3,4•].

Continuous yeast cultures can exhibit oscillations of a much longer period, which are related to the asymmetric nature of the budding cell cycle. Normally random variations in cellular metabolism and cell-cycle regulation produce a heterogeneous population in which individual cells are dispersed throughout the cell cycle. Under aerobic and glucose-limited growth environments, a synchronization mechanism yet to be fully understood causes cell subpopulations to move simultaneously through the cell cycle and leads to sustained oscillations in extracellular measurements [1,5].

Secretion rates of microbial products can be affected by the cell-cycle position of individual cells and therefore on the degree of heterogeneity. Synchronous cultures of the budding yeast *Saccharomyces cerevisiae* have been used to investigate the secretion rates of various proteins as a function of the cell-cycle phase. Studies have shown that significant protein secretion rates are obtained only as the synchronized cells approach mitosis [6,7].

To develop fundamental understanding of cell heterogeneities and their effects on microbial population dynamics, biochemical analysis methods which provide information at the single-cell level are required. Flow cytometry has emerged as a very powerful method for measuring the distribution of cellular properties across large cell populations. By combining cell staining techniques and analysis of light scattering and fluorescence signals, individual cells can be differentiated with respect to their size, protein content, DNA content and other intracellular properties [8]. Recently, flow cytometry has been combined with flow injection techniques to produce automated systems that provide on-line measurements of cell distribution properties [9]. When combined with suitable cell population models, on-line flow cytometry will enable the development of computer-based systems that provide real-time monitoring and control of cellular distributions in microbial fermentations.

This review focuses on dynamic models of microbial populations that explicitly account for cell heterogeneities. Two general approaches are discussed: population

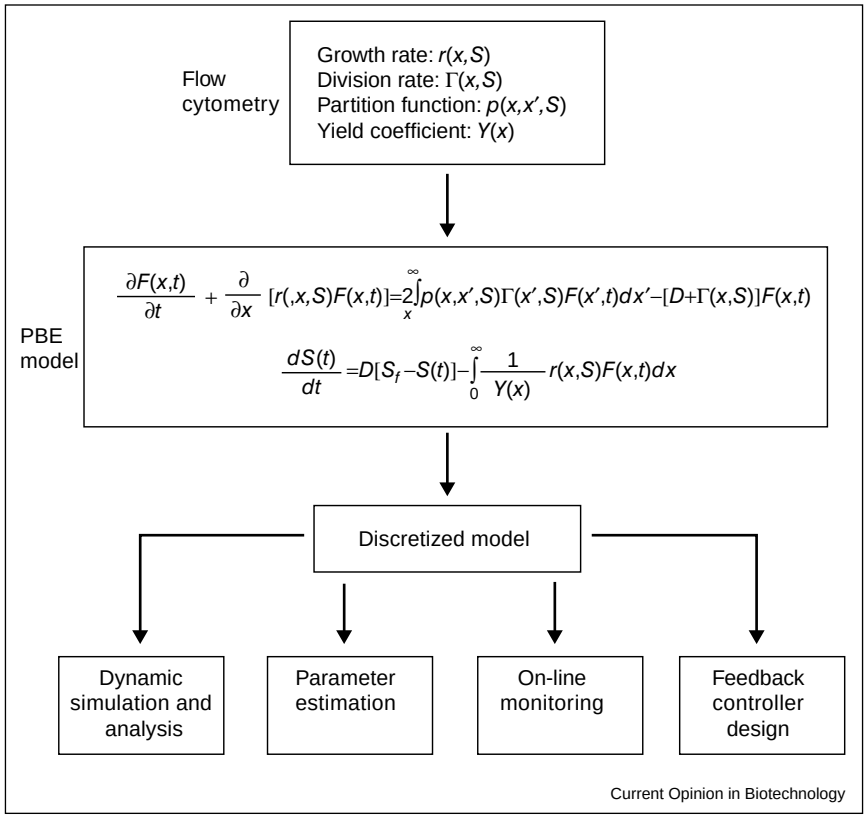
balance equation (PBE) models, in which the intracellular state is characterized by a single variable such as cell age or mass, and cell ensemble models constructed from single-cell models that have more detailed descriptions of cellular metabolism and/or cell-cycle progression. Applications of these cell population models for predicting culture dynamics and designing feedback control strategies are described. Discussion of other types of microbial cell population models [10,11] is omitted for the sake of brevity.

Population balance equation models

The most rigorous approach for describing the effects of cell heterogeneities on microbial culture dynamics is based on the PBE. As originally shown by Fredrickson and co-workers [12], the PBE results from a dynamic cell balance that includes single cell descriptions of cellular growth and division. Individual cells are differentiated using appropriately chosen variables that characterize the intracellular state. PBE models based on a single internal state such as cell age [13] or cell mass [14] are most common due to their simplicity. Typically the PBE is coupled to mass balance equations for the important nutrients in the extracellular environment. A single rate-limiting substrate often is assumed for simplicity [15]. More general formulations that include multistaged descriptions of the cell cycle are also available [16].

Figure 1 shows the basic principles of PBE modeling when a single internal state and a single rate-limiting substrate are employed. The PBE is a nonlinear partial differential equation with two independent variables (time and the internal state) and a dependent variable, which represents the number distribution of cells. Under certain conditions the physiological functions in the cell and substrate balance equations can be extracted from flow cytometric data [17,18]. A wide variety of discretization techniques has been proposed for approximating the

Figure 1



Basic principles of cell PBE modeling. A single variable (x) is used to characterize the intracellular state and a single rate-limiting nutrient (S) is used to describe the extracellular environment. Flow cytometric data can be used to estimate the dependence of the following physiological functions on the intracellular state: the single-cell growth rate (r); the division rate (Γ); the partition function (p), which determines the partitioning of cellular material between two cells resulting from division; and the yield coefficient (Y). The dependent variables of the PBE model are the cell number distribution function (F) and the substrate concentration (S), where $F(x,t)dx$ represents the number of cells per unit volume at time t with intracellular state in the range $[x,x+dx]$. Derivative and integral expressions involving the intracellular state (x) are approximated to yield a nonlinear ordinary differential equation model with time (t) as the only independent variable. The discretized model can be integrated numerically to yield dynamic predictions. It can also be used to estimate unknown model parameters and/or state variables from experimental data and can be utilized to design feedback controllers in which the dilution rate (D) and the feed substrate concentration (S_f) are potential input variables.

Table 1

Recent applications of cell population balance equation modeling.

Microorganism	Problem	Focus	Contribution	Ref
General*	Physiological functions	Modeling	Formulation of PBE model that does not require physiological functions for transitions between cell-cycle phases and in which the division rate is independent of the intracellular state	[20]
S. cerevisiae	Cell-cycle-dependent oscillations	Modeling and parameter estimation	Formulation of mass-structured PBE model with a structured description of the extracellular environment and estimation of physiological function parameters from simulated and experimental data	[21••]
General*	Batch and continuous fermentors	Numerical solution	Detailed study of finite difference discretization methods for numerical solution of PBE models	[22•]
General*	Batch and continuous fermentors	Numerical solution	Detailed study of spectral discretization methods for numerical solution of PBE models	[23]
General*	Batch and continuous fermentors	Numerical solution	Detailed study of finite element discretization methods for numerical solution of PBE models	[24]
S. cerevisiae	Cell-cycle-dependent oscillations	Modeling and control	Formulation of mass-structured PBE model and design of a model-based controller that allows direct control of the cell mass distribution	[25]
S. cerevisiae	Cell-cycle-dependent oscillations	Dynamic analysis	Use of age-structured PBE model to study the dynamic behavior of oscillating yeast cultures	[26]
S. cerevisiae	Cell-cycle-dependent oscillations	Dynamic analysis and control	Use of mass-structured PBE model to study the bifurcations leading to sustained oscillations and to design model-based controllers for oscillation attenuation	[27•]
S. cerevisiae	Cell-cycle-dependent oscillations	Dynamic analysis	Application of nonlinear order reduction to derive simplified nonlinear models which capture the oscillatory dynamics of a mass-structured PBE model	[28]
Yeast	Chemostat	Modeling and control	Formulation of PBE model with two cell-cycle phases and model-based control of product formed only during second cell-cycle phase	[29•]
E. coli	Plasmid instability	Modeling	Formulation and experimental evaluation of PBE model in which the intracellular state is the number of plasmids per cell	[30]
Yeast	Chemostat	Review	Overview of recent progress in dynamic analysis and model-based control of chemostats described by mass-structured PBE models	[44]
S. cerevisiae	Cell-cycle-dependent oscillations	Modeling and control	Overview of author's recent work on mass-structured PBE models and model-based control of oscillating yeast cultures	[45]

*General, no specific type or class of microorganism is considered.

PBE model with a set of nonlinear ordinary differential equations that are suitable for numerical integration. The discretized model can be utilized for dynamic simulation and analysis, for the estimation of unknown model parameters from experimental data, and for the development of on-line fermentor monitoring and control schemes.

Table 1 lists some recent applications of PBE modeling to microbial cell populations. Most studies focus on mass-structured models in which the internal state is characterized by the individual cell mass. Many papers have a significant emphasis on fermentor control. A widely recognized shortcoming of the PBE modeling framework is the lack of a fundamental biochemical basis for selecting the physiological functions associated with single cell growth and division [19]. A partial solution to this problem has been obtained by formulating a PBE in which the division rate is independent of the intracellular state, and physiological functions for transitions between cell-cycle phases are not needed [20]. A complementary approach is to estimate unknown parameters of the physiological functions directly from experimental data. By formulating a mass-structured model with a simple description of the abiotic environment, nonlinear optimization techniques were used to determine parameter values that minimize the least-squares difference between the model predictions and readily available extracellular measurements [21••]. The resulting model accurately predicted the amplitude and period of cell-cycle-dependent oscillations in continuous cultures of *S. cerevisiae*.

A typical PBE model is comprised of a coupled set of nonlinear integro-ordinary differential and integro-partial differential equations. A potentially large set of nonlinear ordinary differential equations suitable for numerical integration is obtained by discretizing the partial derivatives and integrals involving the intracellular state variables. A comprehensive study of alternative discretization methods has been presented in a series of three papers [22•,23,24]. Although considerably more sophisticated techniques were investigated, a relatively simple finite difference approximation scheme was found to provide the best tradeoff between solution accuracy and computational efficiency when applied to PBE models with

single variable and multivariable intracellular state vectors. In another study, discretization using orthogonal collocation on finite elements was found to provide efficient and robust solution of a mass-structured model [25].

Numerical solution codes allow the dynamic behavior of PBE models to be explored and compared with experimental data. Dynamic simulation of an age-structured model for yeast cell-cycle-dependent oscillations has been used to investigate the relationship between the cell age distribution and oscillatory behavior [26]. A complementary approach involves the use of numerical bifurcation techniques to explore the effects of key parameters on long-term model predictions. A bifurcation represents a qualitative change in the dynamic system behavior (e.g. a stable steady-state solution is replaced by a stable oscillatory solution). Bifurcation analysis of a mass-structured PBE model demonstrated the presence of multiple stable states as observed experimentally in continuous cultures of *S. cerevisiae* [27•]. The accuracy of simplified dynamic models derived from a mass-structured PBE model using nonlinear order reduction techniques has been evaluated using bifurcation analysis [28].

The availability of discretized PBE models and/or low-order approximate models facilitates the design of feedback controllers, which provide direct regulation of cell distribution related variables. Recent studies have focused on chemostats where the dilution rate and the feed concentration of the rate-limiting substrate are potential manipulated variables. A model-based nonlinear controller for optimizing chemostat productivity was designed from a mass-structured PBE model with a multistaged cell-cycle description [29•]. A mass-structured model of yeast cell-cycle-dependent oscillations has been used to design simple nonlinear controllers for oscillation attenuation [27•] and optimization-based linear controllers that have the potential to increase the production of key metabolites only synthesized during part of the cell cycle [25]. These studies demonstrate the intimate relationship between the cell-cycle model dynamics and the resulting feedback control strategy. Although not extensively investigated, individual cell metabolism and population dynamics also can be expected to have a major impact on model-based controller design.

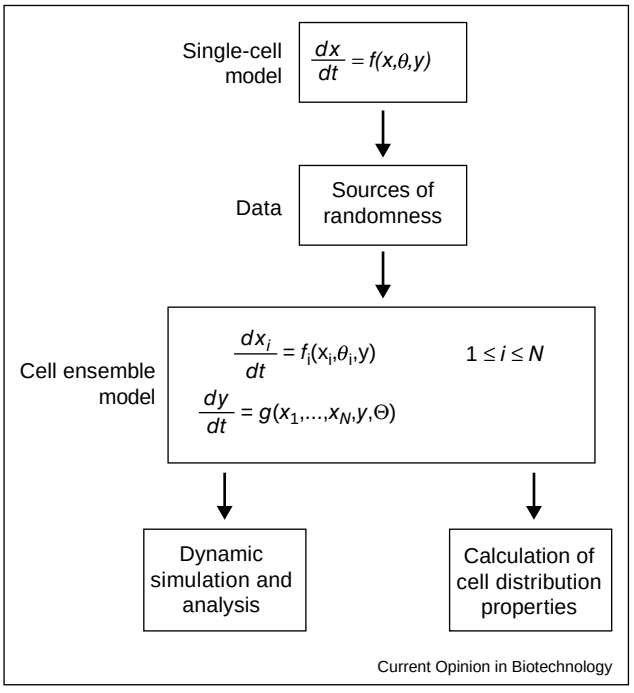
All the PBE models discussed above use either cell mass or cell age to discriminate between individual cells. A PBE model of plasmid instability in continuous *Escherichia coli* cultures utilizes the relative copy number of plasmid DNA as the internal variable [30]. PBE models are closely related to morphologically structured models of filamentous microorganisms, which have a finite number of morphological states. Mathematically the two types of models are equivalent in the limit as the number of distinct morphological states approaches infinity [31]. Recently a morphologically structured model for penicillin production in fed-batch cultures was developed and used to investigate optimal glucose feeding policies [32]. A recently developed model for quorum sensing in bacteria which differentiates upregulated and downregulated cell populations [33] can be viewed as a morphologically structured model with two cell classes. These problems demonstrate the wide applicability of the PBE modeling framework and suggest that many potential applications remain unexplored.

Cell ensemble models

A disadvantage of the PBE modeling framework is the lack of a fundamental biochemical basis for determining the physiological functions associated with single cell growth and division [19]. The incorporation of intracellular reaction pathways which provide mechanistic descriptions of these cellular processes is possible if the distribution function represents the mass fraction (rather than the number) of cells with a particular internal state [31]. In this formulation, the intracellular state is represented by a potentially high dimensional vector, which contains the concentrations of the various intracellular species. In addition to difficulties associated with modeling cell-cycle events, such metabolically structured PBE models are expected to be computationally intractable because discretization will produce a very large number (1 000 000 or more depending on the metabolic description) of nonlinear ordinary differential equations.

Figure 2 shows the basic principles of an alternative cell population modeling approach, which is termed cell ensemble modeling in this review. A dynamic model of a single microbial cell that adequately describes the cellular processes of interest is required [34]. The state vector of this nonlinear ordinary differential equation model contains the concentrations of various intracellular species. An ensemble is constructed from a potentially large number of single-cell models in which intracellular parameters are randomly perturbed from their nominal values to capture heterogeneities associated with particular cellular processes [35]. Typically the random perturbations are assumed to be normally distributed with zero mean and variance consistent with any available data. The cell ensemble model is completed by adding differential equations, which capture the concentration dynamics of the relevant extracellular species. Numerical integration of the ensemble model allows individual cell dynamics and the average dynamics of the cell population to be predicted. Simulation data can be used to compute dynamic cell distributions with respect to any intracellular property predicted by the single-cell model [36]. The goal is to utilize a sufficiently large number of single-cell models such that ensemble averages are independent of the cell number and the computed cell distributions are acceptably smooth.

Figure 2



Basic principles of cell ensemble modeling. The intracellular state vector (x) of the single-cell model contains the concentrations of various species involved in energy metabolism, biosynthesis, cell-cycle progression and other cellular processes. The intracellular parameter vector (θ) includes constants associated with reaction and transport processes. The extracellular state vector (y) contains the concentrations of substrates, secreted metabolites and other species in the extracellular environment. Experimental techniques such as flow cytometry are used to identify intracellular processes that are subject to significant variations between individual cells and to estimate statistical variations of the associated single-cell model parameters. The cell ensemble model is comprised of N individual cell models and extracellular balances, which involve the state vector of each cell (x_i) and a vector of extracellular parameters (Θ). The ensemble model can be used to generate predictions of cell population dynamics and to yield the simulation data required to compute approximate cell number distributions.

The cell ensemble modeling approach was originally proposed by Shuler and co-workers [35,36]. They used ensembles of approximately 225 individual cells to predict steady-state and dynamic size distributions for aerobic and anaerobic cultures of *E. coli* as well as plasmid instability in a genetically modified *E. coli* strain. A list of more recent applications of ensemble modeling to microbial cell populations is shown in Table 2. Most studies focus on the synchronization of single cell glycolytic oscillations observed in specially prepared suspensions of yeasts such as *S. cerevisiae* [3]. The simplest model is based on a single cell description that accounts only for glucose and ATP dynamics and utilizes an ensemble of just two cells which interact via linear couplings [37].

Ensemble models which include more detailed descriptions of the glycolytic pathway and more physiologically based cellular interactions have been proposed. A single-cell model that accounts for the dynamics of four glycolytic intermediates, ATP and NADH has been used to construct ensembles in which cellular interactions are mediated by acetaldehyde secreted into the extracellular environment [38]. Detailed bifurcation analysis shows that a two-cell ensemble model is capable of producing a wide range of periodic solutions including synchronous oscillations, as observed experimentally. An extended version of this cell model which includes the dynamics of two additional glycolytic intermediates has been used to construct a two-cell ensemble for studying synchronization dynamics [39]. A very detailed cell model that accounts for the transport of glucose, glycogen, ethanol, acetaldehyde and cyanide across the cell membrane, the degradation of acetaldehyde by cyanide, the storage of energy as ATP, basic cellular processes consuming ATP and 11 reactions between the glycolytic intermediates was recently proposed [40]. Although the single-cell model provides good agreement with available data, an ensemble model comprised of two individual cells and the necessary extracellular balances does not produce synchronous oscillations. This demonstrates that incorporating more metabolic structure into the single-cell model does not necessarily improve the predictions of the resulting ensemble model.

Much larger cell ensemble models have been constructed to investigate the synchronization of yeast glycolytic oscillations. A simplified cell model, which captures the dominant dynamics of a metabolically structured model [40] near the bifurcation point where sustained oscillations appear, has been derived through the application of nonlinear dynamic analysis techniques [4•]. An ensemble model comprised of 1000 simplified cells was able to capture the rapid synchronization that is observed experimentally upon mixing two cell populations oscillating 180 degrees out of phase. The single-cell model of Wolf and Heinrich [38] has been used to construct ensembles of 1000 cells in which the initial state and/or kinetic parameters of each cell were randomly perturbed from their nominal values [41••]. Synchronous oscillations were obtained for large variations in the initial intracellular state, while much smaller variations in the intracellular reaction kinetics resulted in desynchronization. Dynamic simulation data were used to compute the ensemble averaged NADH concentration as well as the NADH number distribution.

Other applications of the cell ensemble modeling approach have been investigated. A very simple cell model which provides a phenomenological description of biomass accumulation has been used to construct very large ensembles with as many as 100 000 cells for the prediction of average biomass concentration in activated sludge processes [42]. A single-cell model based on a lumped description of cellular growth and proliferation

Table 2

Recent applications of cell ensemble modeling.

Microorganism	Problem	Ensemble size	Contribution	Ref
S. cerevisiae	Glycolytic oscillations	1000	Use of an approximate cell model derived from a detailed single-cell model to construct ensemble models for study of cell population synchronization	[4•]
S. cerevisiae	Glycolytic oscillations	2	Formulation of a very simple ensemble model that includes glucose and ATP dynamics for investigation of cell population synchronization	[37]
Yeast	Glycolytic oscillations	2	Formulation and detailed bifurcation analysis of an ensemble model which includes the intracellular dynamics of ATP, NADH and four metabolites	[38]
Yeast	Glycolytic oscillations	2	Formulation and dynamic analysis of an extended version of the ensemble model in [38], which includes two additional glycolytic intermediates	[39]
Yeast	Glycolytic oscillations	2	Use of a detailed single-cell model to construct an ensemble model that fails to exhibit cell population synchronization	[40]
Yeast	Glycolytic oscillations	1000	Construction of ensemble models for investigation of the effects of random Single-cell variations on cell population synchronization	[41••]
Heterotrophic bacteria	Activated sludge dynamics	100 000	Use of a very simple cell model for biomass accumulation to construct an ensemble model for prediction of average biomass concentration	[42]
Bacteria	Colony growth and metabolic oscillations	Time varying (1–69 000)	Formulation of ensemble models to investigate the effects of random single-cell variations on colony growth and metabolic oscillations	[43]

was used to construct ensembles for the prediction of biomass distributions in bacterial colonies [43]. A shortcoming of the modeling approach proposed by Ginovart and colleagues [43] is that the number of single-cell models is not fixed, but rather increases exponentially with time due to cell division. A computationally tractable alternative is to utilize a fixed number of cell models, each of which represents several individual cells, which varies with time as new cells are created through division [35]. Regardless of their limitations, these studies demonstrate that the cell ensemble modeling approach can be employed whenever an appropriate single-cell model is available. This desirable property suggests that many potential applications remain unexplored.

Conclusions

Table 3 provides a comparison of the PBE and ensemble approaches to cell population modeling. Although the basic strategies employed are different, both techniques allow the description of heterogeneities at the single-cell level. The major distinction is the relative tradeoff between the amount of intracellular structure and the number of cells included. The PBE modeling technique is not limited with regard to cell number, but the intracellular state dimension must be restricted to a few variables to ensure computational feasibility of the discretized model. By contrast, the cell ensemble technique allows direct incorporation of arbitrarily complex single-cell models at the expense of relatively small cell

Table 3

Comparison of cell population modeling techniques.

Modeling issues	PBE models	Ensemble models
Intracellular state	Limited to 1–2 variables	High dimension possible
Number of cells	Infinite	Limited by intracellular state dimension
Single-cell models	Cannot be used	Can be incorporated directly
Heterogeneity	Realized via physiological functions	Realized via single-cell model parameters
Parameter estimation analysis issues	Physiological function parameters	Single-cell model parameters
Numerical solution	Discretization of intracellular state followed by numerical integration	Numerical integration
Dynamic simulation	Prohibitively slow for high intracellular state dimension	Prohibitively slow for large number of cells
Intracellular distributions	High resolution of multidimensional distributions possible	Resolution limited by number of cells
Bifurcation analysis	Applied to mass and age-structured models	Applied to very small ensembles
Feedback controller design applications	Applied to mass-structured models	Unexplored
Batch fermentor dynamics	Several studies	None
Chemostat dynamics	Many studies	Several studies
Yeast glycolytic oscillations	None	Many studies
Yeast cell-cycle-dependent oscillations	Many studies	None
Plasmid instability	One study	One study

numbers, which can limit the resolution of intracellular distributions. Direct comparisons are not currently possible because the two techniques have been applied to different problems.

The combination of increasingly powerful computers and sophisticated numerical algorithms will facilitate the continuing development of microbial cell population modeling. Although recent progress has been significant, several challenges must be addressed before cell population modeling can be viewed as a general purpose tool for biochemical systems analysis. Several issues are particularly important for the PBE modeling technique: the formulation of dynamic models with multidimensional intracellular state vectors; the development of systematic techniques for constructing the physiological functions from flow cytometric data; and the development of discretization and numerical solution techniques that allow efficient simulation of multidimensional models. Corresponding issues for the cell ensemble modeling technique are the development of systematic techniques for introducing heterogeneity into the single-cell models, efficient dynamic simulation of very large ensembles comprised of highly structured single-cell models, the development of more sophisticated methods for computing intracellular distributions from ensemble simulation data, and the incorporation of large ensemble models in dynamic analysis and feedback control strategies.

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