BIOMEDICAL ENGINEERING

Population Balances in Biomedical Engineering

Segregation Through the Distribution of Cell States

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Chapter

1

Introduction

This chapter aims to clarify the concept of *population balance model* or *population balance equation*, terms that are used almost interchangeably in this book. This is followed by a short narrative of the strengths and weaknesses of these models.

1.1 What Are Population Balance Models?

Population balance is not a well-defined concept in science and engineering, but means slightly different things to different people. During the fall of 2004, a Web search on the term "population balance model" gave more than 1 million hits, and a casual perusal of some of the Web pages obtained in this search makes clear this confusion of connotations. In this book, population balance models will connote the equations or sets of equations that model the dynamics of the distribution of states of a population of cells or particles.

Population balances are models describing how the number of individuals in a population and their properties change with time and with the conditions of growth. In engineering, population balances are used to model not just populations of living cells, but also populations of inanimate particles, such as the size and number of crystals in a crystalizer or the size, number, and composition of droplets in an aerosol.

Although an engineering concept, there is a population balance notion that is known to most people and that is the population pyramid. Age pyramids are histograms depicting the number of people in each of a set of age classes. Often, these histograms are split into two parts, one for males and one for females, and are placed with a common vertical

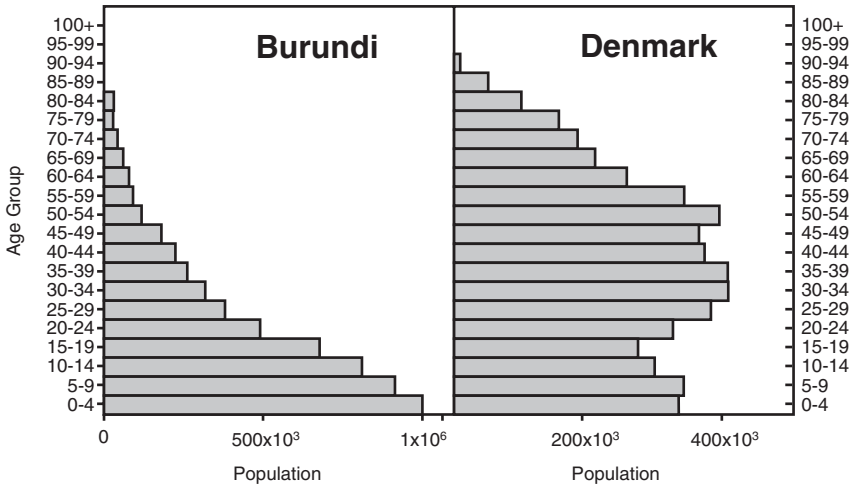

Figure 1.1 Population pyramids for Burundi and Denmark, 2000. (*Source: U.S. Census Bureau.*)

axis signifying age, and two horizontal axes, running in opposite direrctions for males and females, indicating number of individuals in each age class. This placement gives rise to a roughly triangular shape reminiscent of a pyramid, thus the name. The age pyramids for Burundi and Denmark for the year 2000 are shown in Fig. 1.1.

Without knowing anything about the mathematics of population balance models, most people will be able to look at these two pyramids and immediately conclude that

- The population of Burundi is increasing while the population of Denmark is not, or if so, only very slowly compared to the population of Burundi.
- Denmark experienced a baby boom after World War II while Burundi did not.
- The average life span in Denmark is longer than the average life span in Burundi.

The rate of population increase in Burundi can be inferred from the large number of people in the younger age groups as compared to the older groups, indicating a population with a large fraction of young individuals. This trend could conceivably be explained by a high rate of death for all of the age groups, but it is not a valid explanation in this case, since natural death in humans occurs predominantly at older ages. Instead, the large fraction of young people is a result of a high birth rate causing each generation to be larger than the previous and

thus the total population to increase with time. This trend turns out to hold for microbial populations as well: the higher the specific growth rate of the population, the larger the fraction of younger cells and vice versa. The population pyramid for Denmark, on the other hand, shows an approximately constant population size for age groups younger than 60. Only after this age does death cause a significant decrease in population size with age.

The Danish population pyramid is at its widest between ages 25 to 54; the age distribution has a local maximum in this interval of ages. This, of course, is a signature of the baby boom, the increase in birth rate that occurred in most of the western world after World War II, which was a period during which people postponed starting families. Although the Danish population pyramid indicates a population that is not changing rapidly in size, the baby boom hump shows that the age distribution in the population is not at a steady state. The baby boom subpopulation in the western world will, as time goes by, shift toward older ages, resulting in a population with a high fraction of senior citizens and giving rise to concerns about how society can cope with this increase in retirees. This connection between a temporary increase in birth rate and a local peak in the age distribution is also seen in the age distribution of microbial cultures. When such a peak is formed, the culture is said to be synchronized, or partially synchronized, and the sharper the peak in the age distribution, the higher the degree of synchrony is said to be.

The average age in Burundi and Denmark can be easily be calculated from the values of their respective population pyramids. The average age is simply the first moment of the age distribution, and the lower average age for Burundi as compared to Denmark reflects both a shorter life span and a more rapidly increasing population in Burundi.

Population balance models of the populations in Burundi and Denmark will allow for quantitative predictions about the future of the populations in the two countries rather than just the simple qualitative statements above. For instance, models would allow one to predict or estimate future population sizes in Burundi or the fraction of retirees in Denmark, both estimates that are valuable for reaching political decisions about how to manage future changes in the populations. However, the focus of this book is not on models of human populations but of models of cultures of cells, be they single-celled procaryotes, eucaryotes, or even the cells that make up tissues.

Most growth models of cell cultures can be classified as either structured or unstructured, and as distributed or segregated [94]. The term “structured model” refers to a model where more than one variable is used to specify the composition of the biophase. Typically, these

variables are the chemical compounds of the biophase. To keep the number of model variables manageable, models make frequent use of pseudocomponents, functionally similar compounds that have been lumped into groups such as proteins, various types of RNAs, and lipid content. Unstructured models, on the other hand, characterize the biophase by a single variable such as the amount of biomass. Distributed models are models that make the simplifying assumption that the cells in a culture form a single well-mixed biophase, while segregated models are more realistic and take into account the fact that the biological material is segregated into individual cells that are not necessarily identical in composition. In segregated models, the biophase is described by a *distribution of cell states*, a frequency function that indicates the probability that a cell, picked at random, is in a specified state. This specific state can be any measure of the cell state: cell size, cell mass, cell age, DNA content, protein content, etc. The state of a cell can even be specified by using multiple variables such as DNA and protein content, in which case the distribution of states becomes a multidimensional frequency function.

Distributed models can be either structured or unstructured. An unstructured, distributed model consists of a balance on the biomass coupled with mass balances on the media component, and these balances form a set of coupled, ordinary differential equations. A structured, distributed model also consists of coupled ordinary differential equations, balances on the components in the biophase and balances on components in the media—identical to the balances one would write on any two-phase reactor.

Segregated models can be either structured or unstructured, depending on how many parameters are used to describe the state of a cell. They are usually much more complex than distributed models, typically consisting of partial differential, integral equations for the distribution of cell states, coupled to mass balances on the substrate components. Segregated models are a type of population balance model, but the concept of population balances encompasses many more systems than just cell cultures.

The population balance models that are the topic of this book are segregated models of microbial populations. They are not only age distribution models, but also models of the size or mass distribution, or multidimensional models involving several cell state parameters. As alluded to earlier, these models share some of the features and issues of models of human populations. To model either type of population, one will want to know when reproduction or cell division occurs, at what rate cells or individuals in different states die, the state (e.g., size or mass) of newborn cells, and the growth rate of individual cells. Of

course, for the age distribution problem, the last two issues are trivial; newborn cells have age zero and the age growth rate is unity. When other state parameters such as cell mass are used, it is more difficult to say something about the rate of growth of individual cells or the distribution of states of newborn cells.

1.2 The Distribution of States

The models of microbial populations that we will consider here will not be of the discretized version that is exhibited by the human population histogram in Fig. 1.1, but will assume that the state parameter (age, mass, etc.) is a continuous variable, giving rise to distributions of states that are usually smooth functions instead of the discontinuous bins that the histogram represents. (Of course, a smooth distribution can always be represented by a histogram if so desired.) The distributions of states can be scaled several ways, either as a frequency function such that the zeroth moment equals unity, or as a cell number distribution such that the zeroth moment equals the cell number concentration. We will adopt the nomenclature that $f(\cdot)$ indicates the normalized distribution of states and $W(\cdot)$ the cell number concentration distribution of states. Thus, if the state of a cell is given by z, then

$$f(z, t)dz = \text{fraction of cells with state } z \in [z, z + dz]$$

at time t and similarly

$$W(z, t)dz = \text{cell number concentration of cells with state } z \in [z, z + dz]$$

The two distributions scale such that

$$\int_z f(z, t)dz = 1$$

where the z subscript in the integral indicates that the integration is over all possible cell states z. Similarly

$$\int_z W(z, t)dz = N(t)$$

where $N(t)$ is the cell number concentration at time t. Clearly,

$$W(z, t) = N(t)f(z, t)$$

and the equations that describe how these functions evolve with time and under different growth conditions are the population balance

models that we seek. The fact that these distributions indicate that the number of individuals in a given group can be a fractional number does not contradict the fact that in real populations the number of individuals within a given group is always an integer because the distributions should be thought of in a statistical sense. They represent the probability that a cell chosen at random is in a given group or interval of states. Also, in most practical applications, the number of cells in a population is so huge that the difference between the true discrete population and the continuum approximation represented by the distribution of states becomes negligible.

Often one may want to find several different distributions of states for the same population. For instance, one may want to know both the distribution of cell mass and the distribution of cell age. Instead of solving for each distribution separately, one can, since a single state parameter is used, solve for either one and find the other by a variable transformation. For instance, consider a case where the age distribution is known and where the mass distribution is desired. All we need to know to carry out the transformation is the cell mass as a function of cell age. Call this function $m(a)$ and the inverse function $a(m)$; then

$$\text{Number of cells between } a \text{ and } a + da = f(a)da$$

$$\text{Number of cells between } m(a) \text{ and } m(a + da) = f(m)dm$$

and thus

$$f(a)da = f(m)dm \Rightarrow$$

$$f(m) = f(a(m))\frac{da}{dm}, \quad f(a) = f(m(a))\frac{dm}{da}$$

The distribution of states can be partially characterized by various scalar quantities such as the zeroth moment mentioned above. In general, the nth moment of $f(z, t)$ is

$$M_n(t) = \int_z z^n f(z, t)dz = \frac{\int_z z^n W(z, t)\,dz}{\int_z W(z, t)\,dz}$$

The first moment has a simple biological interpretation; it is the mean or average z value of the cells in the population, e.g., the average cell mass or cell size. The moments defined this way are mathematically important because an approximate distribution can often be reconstructed from the moments. However, in terms of descriptive value, the centered moments are preferred. These are defined as

$$\mathcal{M}_n = \int_z (z - M_1)^n f(z, t)\, dz$$

and many of these have common names such as the second centered moment or the variance σ^2,

$$\sigma^2 = \int_z (z - M_1)^2 f(z, t)\, dz = M_2 - M_1^2$$

which describes how broad or uniform the distribution is. For a perfectly synchronized distribution in which all cells are in the same cell state, the variance equals zero. The asymmetry of the distribution is measured by the skewness defined as

$$\gamma_1 = \int_z (z - M_1)^3 f(z, t)\, dz/\sigma^3 = \frac{M_3 - 3M_1M_2 + 2M_3^3}{(M_2 - M_1^2)^{3/2}}$$

The reason for division by σ^3 is that it renders the skewness dimensionless. If a distribution is symmetric, it has zero skewness; if it has a tail at values greater than its maximum, it has positive skewness; if the tail is at values less than the maximum, it has negative skewness. Finally, the kurtosis is defined in terms of the fourth centered moment as

$$\gamma_2 = \int_z (z - M_1)^4 f(z, t)\, dz \,/\, \sigma^4 - 3 = \frac{M_4 - 4M_1M_3 + 6M_1^2M_2 - 3M_1^4}{M_2^2 - 2M_1^2M_2 + M_1^4} - 3$$

The reason for the –3 term in the definition is that it results in the normal distribution having a kurtosis of 0. The kurtosis defined above is therefore sometimes called the *kurtosis excess*, as opposed to the *kurtosis proper*, which is defined without the –3 term. The kurtosis is a measure of the degree of peakedness of a distribution. If the distribution is more concentrated around the mean than the normal distribution, then the kurtosis is positive, otherwise it is negative.

1.3 The Age Population Balance

Derivation of the age population balance is particularly easy and will be done first to illustrate the general concept of a particle balance. We can obtain the equation by doing a cell number balance on a group of cells with ages between b and c, where we assume $0 < b < c$. The age bracket that defines the cells is an example of a so-called *control volume*, the "volume" in state space over which a number balance, or any

other kind of conservation balance for that matter, can be written. The number of cells in the control volume is

$$\int_b^c W(a,\, t)\, da$$

This number changes with time, and the rate of change in the number of cells inside the control volume is the time derivate of the integral:

$$\text{Rate of change in cell number} = \frac{\partial}{\partial t}\int_b^c W(a,\, t)\, da = \int_b^c \frac{\partial W}{\partial t}\, da$$

The number of cells in the control volume changes through three processes: Cells leave the group as they grow older than c, younger cells enter the group as they grow older than b, and cells leave the group because they divide. The rates at which cells enter and leave the group by growth are $W(b, t)$ and $W(c, t)$, respectively. The rate at which cells of age a divide is harder to account for, and we will need to define a function, $\Gamma(a, t)$, such that $\Gamma(a, t)\ W(a, t)$ equals this rate. Γ is called the division intensity, and we shall return to this function later and discuss it in more detail. Thus, the rate at which cells leave the control volume through division equals the rate for cells of age a integrated over all the control volume ages:

$$\text{Rate of cell leaving by division} = \int_b^c \Gamma(a,\, t) W(a,\, t) da$$

The rate of change of the number of cells in the group can now be related to the rates at which cells enter and leave the group by a number balance:

Rate of change in cell number =

rate of cells entering – rate of cells leaving

or, as an equation,

$$\int_b^c \frac{\partial W}{\partial t} da = W(b,\, t) - W(c,\, t) - \int_b^c \Gamma(a,\, t) da$$

The cell balance is not particularly useful in this form, so we will rewrite it by first writing the difference $W(b, t) - W(c, t)$ as an integral,

$$\int_b^c \frac{\partial W}{\partial t}\, da = -\int_b^c \frac{\partial W}{\partial a} da - \int_b^c \Gamma\,(a,\, t)\, W(a,\, t) da$$

then collecting all the terms under a single integral sign,

$$\int_b^c \left\{ \frac{\partial W}{\partial t} + \frac{\partial W}{\partial a} + \Gamma(a, t) W(a, t) \right\} da = 0$$

As the limits of the integral are arbitrary, the integrand itself must be identically zero, giving the desired result:

$$\frac{\partial W}{\partial t} + \frac{\partial W}{\partial a} = -\Gamma(a, t) W(a, t) \tag{1.1}$$

Since this equation was obtained from a number balance on cells inside a specified age bracket or control volume, this equation (as well as other equations obtained by number balances) will be referred to as a *population balance equation* (PBE). By themselves, population balance equations do not present sufficient information to solve for the distribution of states. They must first be supplied with side conditions or boundary conditions, initial conditions, and typically equations for the concentrations of growth-limiting nutrients in the medium, as well as equations that relate these concentrations to the division intensity and other kinetic functions in the population balance equation. We will refer to the combination of the population balance equation and all its side conditions and supporting equations as a *population balance model* (PBM). The alternative term *corpuscular*[1] *models* has been suggested [81], but the term has never caught on, while the term segregated model is used in many biochemical engineering books for PBMs of cell cultures [3, 10, 66].

1.4 Other PBMs

The term "population balance model" was firmly established as the preferred term when a United Engineering Foundation conference in Kona, Hawaii, in the year 2000 titled itself the Engineering Foundation Conference on Population Balance Modeling and Applications, and when, shortly after this conference, Professor Doraiswami Ramkrishna published the first general textbook on population balances simply entitled *Population Balances* [74]. It is immediately obvious in looking through this book or through the papers from the Kona conference [47] that population balance models are not limited to populations of microbial cells. In fact, in engineering the term refers to any number balance over a particulate system, and population balance models have been formulated for aerosols, crystallizers, emulsions, soot formation, polymerization kinetics, and granulation operations. Even networks

[1]Pertaining to, or composed of, corpuscles, or small particles.

and traffic flow can be modeled with population balance equations. All these models have a similar mathematical structure, and, looking back at the derivation of the age distribution population balance equation above, one should notice that there is nothing in the derivation that is particular to living cells. The very same arguments can be used to formulate a balance equation for crystals that grow and break in a crystallizer. Common to all population balances of this type is that they describe the dynamics of a population of particles in terms of the kinetics of the single particle, i.e., in terms of the growth rate of a single particle, the probability of breakage/division of this particle, and the probability that a newly formed particle is in a certain state. In some particulate systems, additional processes must be considered. For instance, in crystallization, new crystals can be formed, not just by breakage of larger crystals, but also by nucleation, and the population balance for a crystallizer must therefore include a nucleation rate. Similarly, aggregation or agglomeration is an important process that must be included in population balances of aerosols, emulsions, and flocculation processes.

People who work with population balances are often fond of pointing out that particulate systems that are physically dissimilar can all be modeled with PBMs that share a common mathematical structure. Unfortunately, this fondness for pointing out the shared mathematical basis has not resulted in a common nomenclature for PBMs. Each physical system often carries its own nomenclature over into the PBM. This can make it a challenge to read the literature on PBMs from areas outside one's own, but it is a worthwhile effort to undertake if one wants to obtain a firmer grasp of these models. This is particularly important when it comes to computational aspects, the numerical solution and simulation of PBMs, where algorithms that have proved successful for one model can often be applied, with little change, to PBMs for different physical systems.

Population balance models started to appear in the engineering literature in the early '60s, the first being a model of the size distribution of particles in a crystallizer, including nucleation but assuming no breakage of particles [78]. This was followed by a model of the age distribution of viable and nonviable cells in a cell culture [34], and a study of size distributions in two vessel systems when particles can either grow or shrink [4]. It was quickly realized that these models shared a common mathematical structure, and general presentations of abstract population balance models soon appeared [48, 77] as well as more general overview papers of the current state of the art of population balance models [73, 76]. A few text books have also been published, but apart from the book by Professor Ramkrishna [74], these

have had a narrow focus such as crystalization [79] or process control [21]. The introduction to population balances for many of the people working with microbial cultures are arguably two early papers from Professor Arnold Fredrickson's group at the University of Minnesota [27, 33]. Both papers are recommended as excellent introductions to PBMs of cell cultures. The first [27] presents a derivation and analysis of PBMs with mass or age as the state parameter and discusses the relationship between the mass and age models. Also presented are models of single-cell growth rates based on the assumption that uptake of mass is proportional to the cell surface area; spherical (cocci) and cylindrical (bacilli) cells are modeled. The second paper [33] presents a more ambitious derivation and analysis of structured PBMs.

1.4.1 Population balances in ecology

Before concluding this section, it must be pointed out that the term *population balance model* is also used for any number of models, ecological models in particular, that model the size of populations of one or several species. Being primarily concerned with the dynamics of population sizes, they need not employ the concept of a distribution of states at all and can be mathematically quite different from the PBMs described above. For instance, the celebrated Lotka-Volterra model of a predator-prey system consists of two coupled ordinary differential equations [55], while the logistic map is a first-order finite difference equation which has been used to model the number of individuals in successive generations [57].

However, some ecological models, often called *density-dependent population models* or *physiologically structured population models*, do incorporate a distribution of states of the population being modeled. The main difference between the PBMs of particulate systems that are the focus of this book and the physiologically structured models used in ecology is that PBMs of particulate systems typically include equations for the composition of the environment while physiologically structured models do not. The reason for this difference is that credible models exist that describe the effect of the environment on growth of many types of particles, while such models often cannot be identified in ecological modeling. For instance, the Monod model [61], which is often used to model the effect of the limiting substrate concentration on the specific growth rate of a cell population, is a plausible model of the effect of substrate concentration on the growth rate of individual cells, and it is therefore reasonable to include equations for the composition of the medium in a PBM of microbial cells. On the other hand, in ecological models, kinetic terms such as birth or death rates are modeled not as dependent on the composition of the environment, but on various

weighted moments of the distribution of states. This creates a model with a mathematical structure that is superficially similar to that of PBMs but which is nevertheless different, and the literature for PBMs and that for physiologically structured population models therefore do not overlap much. The reader interested in learning more about physiologically structured models can consult the book by Cushing [25].[2]

1.5 PBMs of Cell Cultures

Cell cultures possess various features that make them different from many other particulate systems that are modeled by PBMs and that make it possible to simplify the general form of the population balance. For instance, if one ignores processes such as meiosis and spore formation, cells always split exactly in two at cell division, as opposed to many other particles that can fracture into any number of pieces. And because new cells arise only by division of older cells, PBMs for cell cultures never contain a nucleation term. Additionally, PBMs for cell cultures do not contain a term for aggregation. Granted, mating and conjugation occur in sexual reproduction and cells may aggregate to form cell clumps. But sexual reproduction is not an important process in bioreactors, and, although cell aggregation does create a population balance problem in terms of the distribution of aggregate sizes, this problem is independent of the distribution of cell states unless the aggregation has a strong effect on the growth kinetics of the single cells. These processes have therefore so far been ignored in the population balance models of cell cultures in the literature. It is quite possible, of course, that interesting population balance problems can be identified for cell cultures in which sexual reproduction plays a large role or in which cell clumping is so significant that the growth kinetics of single cells are affected. Finally cells, as opposed to all other kinds of particles that are modeled by population balances, can die. PBMs for cell cultures may therefore contain a term that accounts for cell death.

In addition to the constraints placed by biology on PBMs of cell cultures, there are several simplifying assumptions that are routinely made in writing PBMs for cell cultures. Cells, when growing at their maximum rate, double no faster than about once every 15 minutes, while the mixing times in most bioreactors are of the order of seconds. PBMs of cell cultures therefore assume that the cultures are well mixed and the position and velocities of the cells, so-called external parameters, play no role in the models. Only internal parameters such

[2]Be sure to download errata to the book from the author's website, http://math.arizona.edu/cushing.

as age, size, and concentrations of metabolites are used in the distribution of states.

In summary, the processes that determine the specific form of the PBE for a cell culture are single-cell growth rate, cell division rate, some function specifying how cell matter is distributed at division, and possibly cell death. But these processes are essentially the processes that define the cell cycle. PBMs are therefore closely linked to the concept of the cell cycle, and they provide a mathematical description of the dynamics of the entire cell culture in terms of the dynamics of the individual cells as they pass through their cell cycles.

Population balances of cell cultures have been applied to a wide range of problems [95], and one may well ask when they should be used in preference to other types of models. A vast majority of mathematical models of cell culture dynamics found in the literature are distributed models, models in which all the various metabolite concentrations are averages over all cells in the culture. But average concentrations almost never reproduce the correct kinetics. To see this, start by making the (hopefully) obvious point that there are differences between cells in a culture and consider the contrived but illustrative case in which some fraction of the cells, F, is in one state while all other cells are in a different state. Assume that the two states differ in their intracellular concentrations of a substrate that is enzymatically converted to a product, and assume further that the enzyme obeys Michaelis-Menten kinetics. Then the rate of production formation is found as the sum of the rate of production from the two subpopulations,

$$< Rp > = F\frac{\upsilon_m S_1}{K + S_1} + (1 - F)\frac{\upsilon_m S_2}{K + S_2}$$

where S_1 is the substrate concentration in the first subpopulation and S_2 is the substrate concentration in the second subpopulation. If this process is instead modeled by using a distributed model, then the rate of product formation would be calculated on the basis of the average substrate concentration,

$$Rp(< S >) = \frac{\upsilon_m(FS_1 + (1 - F)S_2)}{K + FS_1 + (1 - F)S_2}$$

These two rates are not the same and a distributed model will therefore fail to accurately predict the true rate of product formation in this system. Population balance models are therefore inherently more correct than distributed models. However, distributed models are excellent models in many cases. The error that is introduced by lumping

of the biophase is negligible in comparison to the errors that result from simplification of the metabolism down to some manageable number of reactions, or the errors that are caused by ignorance of the model parameter's values.

It is somewhat of an art to pick the best type of modeling approach for a given problem, but in the case of PBMs versus distributed models, there are important differences between the approaches that usually make the choice obvious. First of all, PBMs must be used in modeling phenomena that are inherently segregated, that is, phenomena in which the distribution of cells over the cell cycle is important. Foremost among these phenomena is cell cycle synchrony, which cannot be modeled by a distributed model. The growth of tissue and the distribution of cell types in a tissue are also a type of problem that cries out for a population balance model. However, not much work has yet been done on PBMs of tissue cultures. There are very likely interesting problems in PBM modeling of tissue culture that await discovery.

Distributed models are superior to PBMs when a detailed description of the metabolism is required. Distributed models consist of coupled, ordinary differential equations (one equation for each metabolite), and models with hundreds of equations or metabolites can readily be solved on computers. Population balances, on the other hand, cannot yet cope with a detailed description of the metabolism because this requires a large number of cell state variables, i.e., a high dimensional distribution of states, and this makes solution of the model intractable with today's computing power. To see why, consider again the population pyramids in Fig. 1.1. If one uses 10 bins in the histogram, then that requires keeping track of 10 variables. Adding another state variable to the description, individual weight, for instance, and using again 10 bins in the weight histogram, the two-dimensional age-weight histogram will require 10-by-10 bins or 100 bins, or 100 variables to keep track of. Adding yet another state variable brings the number of variables to keep track of to 1000. A description with 100 state variables, a modest number by the standards of distributed models, brings the number of variables to keep track of to 10^{100}, an unmanageable number with today's computing power. Consequently, most population balance models of cell cultures are unstructured and use only a single cell state parameter. It is a disappointing fact that currently (2005 C.E.), detailed simulation of a three-dimensional PBM would be considered cutting-edge work.

Chapter

2

Unstructured PBMs

Unstructured population balance models use a single variable, such as cell mass, to indicate the state of a cell in the culture. Unstructured models are the least complex PBMs, and will be explored in this and the following chapters. We will derive a general population balance model that uses cell mass or any other variable that is conserved in a cell division, together with associated substrate and product balances. The age population balance is also rederived together with the boundary conditions that are specific to cell age as the cell state parameter.

2.1 PBEs with Conserved Cell State Parameter

A state parameter such as cell mass is conserved in a cell division, in the sense that the sum of the mass of the two newly formed cells is equal to that of the cell that divided. All PBEs based on such a conserved cell state parameter share the same general form. Before deriving this model, we must define the physical setting of the cell population a little better. We will consider a culture inside a well-mixed vessel with one liquid feed stream and one liquid exit or product stream. The vessel may also be supplied with a gas feed for aeration and have an exit gas stream. However, as the gas streams do not contain any cells, they can be ignored for the moment. The two liquid streams are assumed to have the same volumetric flow rates and the feed stream is assumed sterile but will contain nutrients required for growth. Because the liquid volume change associated with biochemical reactions usually is insignificant, the volume of the culture can be assumed constant. This type of reactor is usually called a CSTR, short for continously stirred

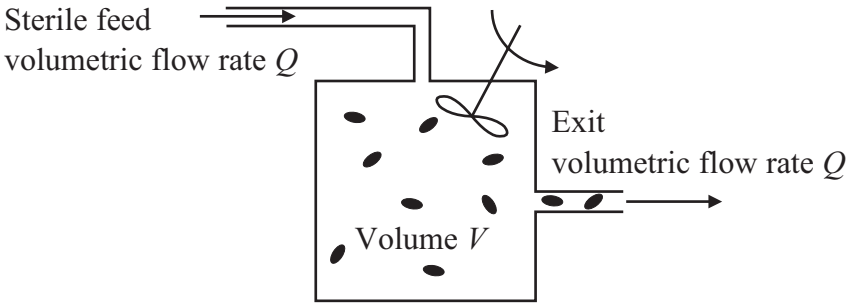

Figure 2.1 Chemostat or CSTR schematic. This idealized reactor type is assumed well mixed, with input and exit streams that have the same volumetric flow rates Q.

tank reactor, or even C*. In the biochemical engineering literature it is often called a chemostat, the term which will be used here. A schematic is shown in Fig. 2.1.

The dilution rate of the reactor is defined as the volumetric flow rate through the vessel divided by the culture volume, $D = Q/V$, and one can easily show that cells in the vessel will wash out of the vessel with the specific rate D. In the absence of any growth processes, cell concentration will therefore decrease exponentially with time as e^{-Dt}. The chemostat model encompasses the batch reactor as the special case where the dilution rate equals zero. Derivation of population balance models for other reactor configurations, such as fed-batch reactors are left as an exercise.

Operation of the chemostat is characterized by its *operating parameters*. These are the parameters one can specify when running the reactor in the plant or in the laboratory. They are the dilution rate and the composition of the feed stream, typically the concentration of the growth limiting nutrients. Many of the models considered later will assume a single growth-limiting substrate with a feed concentration C_{Sf}, giving only two operating parameters, D and C_{Sf}. During steady-state operation, the values of the operating parameters determine the composition of the reactor content and the exit stream and, given a model of the growth kinetics inside the reactor, one can calculate these outlet properties as functions of the values of the operating parameters and the model parameters (in principle, at least). A key objective of this book is to describe how this calculation is done when a PBM is used to model the growth kinetics. In rare cases, a model may allow several steady-state solutions, and in such cases, a more detailed model analysis is required to determine which of the steady-state solutions are stable, and thus experimentally observable. Among the observable solutions, the one that is actually seen in a given situation will depend on how the reactor is "started up." Under transient (time-dependent)

operation, the properties of the exit stream will be functions of the values of the operating parameters, which may now be functions of time themselves, the model parameters, and the initial condition, the state of the reactor at some initial time when the reactor is first started up.

Consider a cell culture in a well-mixed chemostat with a dilution rate D, which may be time dependent, although we will not write this dependence explicitly. Let the cell state parameter be called z, and assume that z is conserved in a division; i.e., it can be cell mass, content of any compound, volume, etc. (but not age). Assume further that z increases as the cell ages. The cell number balance will be done over a differential control volume defined as the cells with states between z and $z + dz$. Cells enter the control volume through growth and birth and leave through growth, division, and possibly death, and by being washed out of the reactor; see Fig. 2.2.

The cell number balance over the control volume now states that

$$\begin{aligned}\text{Rate of cell accumulation} = \\ \text{rate of cell birth} + \text{growth flux in} \\ - \text{growth flux out} - \text{rate of cell division} \\ - \text{rate of cell death} - \text{rate of reactor washout}\end{aligned}$$

The number of cells inside the control volume, per volume of the reactor, is the cell number concentration distribution $W(z, t)$ multiplied by dz, the "size" of the control volume. The rate of accumulation of cells inside the control volume is the time derivative of this term:

$$\text{Accumulation} = \frac{\partial W(z, t) dz}{\partial t}$$

Cell growth is described by the function $r(z)$. This is the single-cell growth rate, the rate of increase in z for a cell in the state z, i.e., the same as dz/dt or equivalently dz/da, where a is cell age. Growth results in two fluxes, one in and one out of the control volume:

$$\text{Growth fluxes, in–out} = r(z)W(z, t) - r(z + dz)W(z + dz, t)$$

The fluxes out of the control volume due to division and due to death of cells inside the volume are described by similar terms. We define the following two functions:

$$\Gamma(z)dt = \text{fraction of cells in state } z \text{ that divide between } t \text{ and } t + dt$$

and

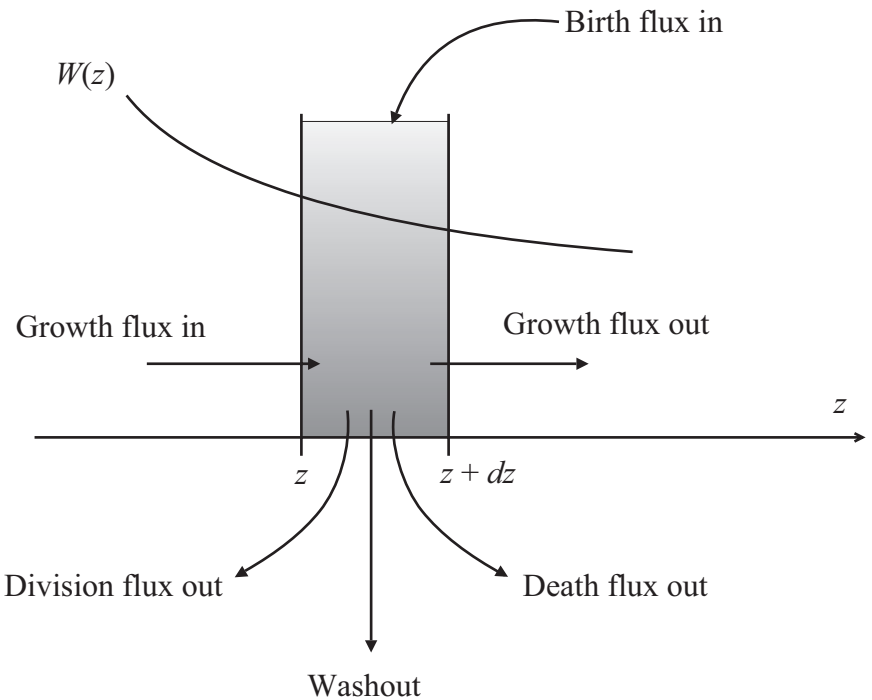

Figure 2.2 Cell fluxes in and out of a differential control volume in state space z, with fluxes indicated. $W(z)$ is the distribution of states.

$$\Theta(z)dt = \text{fraction of cells in state } z \text{ that die between } t \text{ and } t + dt \qquad (2.1)$$

The function $\Gamma(z)$ is called the *division intensity* or *division modulus*, and $\Theta(z)$ is called the *death intensity* or *modulus*. They represent the specific rates of division and death, respectively. Although not written explicitly above, both are functions of growth conditions such as substrate and product concentrations and temperature, and are therefore indirectly functions of time. The control volume fluxes due to division and death are

$$\text{Division and death} = (\Gamma(z) + \Theta(z))W(z, t)\, dz$$

The flux of cells out of the control volume due to washout is

$$\text{Washout flux} = D \cdot W(z, t)\, dz$$

Finally, finding the flux of cells into the control volume by birth will require the use of a distribution of birth states, a function specifying

how cell material is partitioned between the new cells formed in a cell division:

$$p(z, \tilde{z})dz = \text{fraction of newborn cells with a cell state between } z \text{ and } z + dz, \text{ formed by division of a cell in the state } \tilde{z}$$

We can now write the birth flux of cells into the control volume. The rate of births from division of cells in the state $\tilde{z}$ is proportional to the rate of division, $\Gamma(\tilde{z})W(\tilde{z}, t)$. The fraction of these cells that are born into the control volume is proportional to $p(z, \tilde{z})\ dz$. The total birth flux is then obtained by integration over all dividing cells. New cells form only from larger dividing cells, so $p(z, \tilde{z}) = 0$ if $z > \tilde{z}$, and the lower limit on the integration can therefore be written as either z or 0.

$$\text{Flux in by birth} = 2\int_0^\infty \Gamma(\tilde{z})W(\tilde{z}, t)p(z, \tilde{z})dz\ d\tilde{z}$$

The factor of 2 appears because each division results in formation of two new cells. Putting all this together and dividing through by dz gives the population balance equation

$$\frac{\partial W}{\partial t} + \frac{\partial rW}{\partial z} = 2\int_0^\infty p(z, \tilde{z})\Gamma(\tilde{z})W(\tilde{z}, t)\ d\tilde{z} - (D + \Gamma(z) + \Theta(z))W(z, t) \tag{2.2}$$

Notice that this equation is homogeneous, so unless other conditions are invoked, the solution is determined only up to a constant factor. Specifically, the steady-state equation can be divided through by the cell number concentration to obtain a mathematically identical equation for the normalized distribution, $f(z)$. The two functions $\Gamma(z)$ and $p(z, \tilde{z})$ appear in some form in all types of population balances, whether they be balances for cells, crystals, aerosol drops, or some other type of particle, and are called the *breakage functions*.

Equation (2.2) must be supplied with an initial condition and boundary conditions. As new cells cannot grow from nothing, the growth flux from $z = 0$ must be zero:

$$r(0)W(0, t) = 0 \tag{2.3}$$

Physically, this boundary condition states that the nucleation rate is zero in a cell culture. A similar condition, often called a *regularity condition*, is imposed at infinity,

$$r(\infty)W(\infty, t) = 0 \tag{2.4}$$

stating that cells cannot vanish from the system by growing arbitrarily large. In other words, there is no "sink" at infinity. Note that both boundary conditions specify a zero growth flux, not a zero value of the distribution of states.

2.2 Breakage, Death, and Growth Functions

The PBE in Eq. (2.2) contains four functions that shape the distribution of states: death and division intensity, $\Gamma(z)$ and $\Theta(z)$; the distribution of newborn cell sizes, $p(z, \tilde{z})$; and the single-cell growth rate, $r(z)$. Unfortunately, there is little information available that can help guide the choice of expressions used for these functions, and somewhat arbitrary choices for these functions may have to be made. However, it is the essence of good modeling to eschew a detailed description of some of the parts being modeled if the remaining parts of the model cannot support this high level of detail. Considering the substantial simplifying assumptions that are inherent in one-dimensional or unstructured population balances already, it does not make sense to worry too much about the detailed form of these functions, and one should seek functions that, while biologically reasonable, give models that are as easy as possible to work with.

2.2.1 Division intensity Γ

The division intensity Γ is a function of the cell state z and of the concentrations of the substrates in the media. It will be practically zero during the G1 and S phases and rise sharply toward the end of the G2 phase. Faster population growth rates require that the cells divide more often, i.e., at younger ages, and it is thus reasonable to expect that Γ, as a function of cell age, will shift toward younger ages and/or increase more rapidly with age as the population growth rate increases. As population growth rates typically increase with increasing substrate concentrations, Γ must depend on substrate concentrations in such a way that increasing substrate concentrations bring about this shift toward younger ages. Similarly, it is reasonable to expect that the division intensity with respect to cell mass will be close to zero until some critical cell mass is attained, then increase steeply with increasing cell mass.

A suggestion first made by Eakman et al. [27, 28] is to assume that cell mass at division roughly follows a gaussian distribution. An exact gaussian distribution is obviously not possible because cell mass must be nonnegative. Assuming a distribution of division masses of the form

$$h(m) = \frac{2e^{-((m-m_c)/\varepsilon)^2}}{\varepsilon\sqrt{\pi}\left(\text{erf}(m_c/\varepsilon)+1\right)}$$

they showed that the division intensity will be

$$\Gamma(m, C_S) = \frac{2e^{-((m-m_c/\varepsilon))^2}}{\varepsilon\sqrt{\pi}(1-\text{erf}^{((m-m_c)/\varepsilon)})} r(m, C_S)$$

Here ε and m_c are adjustable model parameters and C_S is the substrate concentration. Notice that the substrate dependence only appears as an argument in the factor $r(m, C_S)$, the single cell growth rate. The expression can be rewritten in a compact, dimensionless form as

$$\frac{\Gamma\varepsilon\sqrt{\pi}}{2r} = \frac{e^{-x^2}}{1-\text{erf}(x)} \tag{2.5}$$

where $x = (m - m_c)/\varepsilon$. The graph of this function is shown in Fig. 2.3. The compact form in Eq. (2.3) shows that this model of $\Gamma(m)$ has a limited amount of built-in flexibility. The inherent shape of the function remains the same irrespective of the values of the two parameters ε and m_c, with a value near zero when $m < m_c - 2\varepsilon$ and a rapid increase with m after this point. Evaluating the function for very large arguments can be tricky because, for large arguments, both numerator and denominator go to zero and an accurate evaluation therefore requires a large number of significant digits.

2.2.2 Distribution of birth states *p*

This function describes how cell matter is partitioned between daughter cells at division, and it must be a function of the state of the dividing cell. There is less reason to think that it will be a strong function of medium composition. Several comments can be made about the mathematical properties of the distribution of birth states, $p(z, \tilde{z})$. When z indicates a physical quantity that is conserved in division, such as total cell mass, the newborn cell cannot be born in a state with a larger value of z than the dividing cell. The probability is therefore 1 that the newborn cell will be in a cell state in the interval $[0, \tilde{z}\,]$, or

$$\int_0^{\tilde{z}} p(z, \tilde{z})dz = 1 \tag{2.6}$$

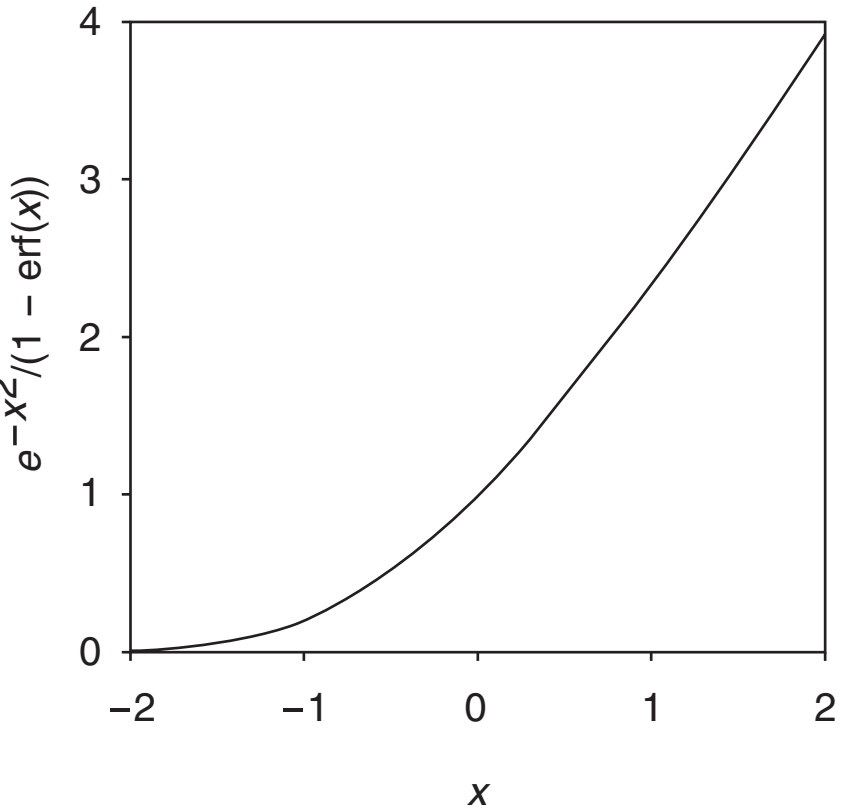

Figure 2.3 Dimensionless division intensity versus dimensionless mass, Eq. (2.5).

Similarly, the cell state of newborn cells must on average equal half that of the dividing cell, $\tilde{z}/2$, and the first moment of $p(z, \tilde{z})$ must therefore equal $\tilde{z}/2$:

$$\int_0^{\tilde{z}} z\, p(z, \tilde{z})\ dz = \frac{\tilde{z}}{2}$$

Finally, $p(z, \tilde{z})$ must satisfy the symmetry condition

$$p(z, \tilde{z}) = p(\tilde{z} - z, \tilde{z})$$

In some organisms, such as budding yeasts, cell matter is distributed unevenly but systematically between the two cells formed in a division. However, lacking such empirical observations, it is reasonable to assume that cell components are distributed at random in a division and the central limit theorem indicates that the mass distribution of newborn cells must be approximately gaussian. Again, cell mass must

be nonnegative, so the gaussian distribution must be truncated at zero and scaled, giving the suggested form for $p(m, \widetilde{m})$ [27, 28]:

$$p(m, \widetilde{m}) = \frac{e^{-((m-\widetilde{m})/2)/\zeta)^2}}{\zeta\sqrt{\pi}(1 - \mathrm{erf}(\widetilde{m}/2\zeta))}$$

The simpler, rational function

$$p(m, \widetilde{m}) = 30\frac{m^2(\widetilde{m} - m)^2}{\widetilde{m}^5}$$

which has all the required properties, has also been suggested [93].

2.2.3 Death intensity Θ

Cell death is clearly a function of the environment, so death intensity should generally depend on the composition of the growth medium. It can also depend on the cell state because cell death may occur predominately in only a part of the cell cycle. For instance, many antibiotics function by inhibiting DNA synthesis, and these antibiotics therefore only kill cells in the S phase, a fact that should be reflected in the choice of function for death intensity. However, barring such known mechanisms of death, there is little reason to assume other than that death occurs uniformly over the cell cycle and that death intensity therefore is independent of cell state. Another possibility, which also gives a simple PBE, is to assume that cell death occurs only at the time of cell division. For instance, if death is modeled by assuming that a fraction Θ of dividing cells die during the division process, then the average number of new cells formed in a division equals $2(1 - \Theta)$ and this factor must be substituted for the factor of 2 in front of the integral term in Eq. (2.2).

However, cell death is an ambiguous term in the context of single-cell organisms. A cell may be considered dead if it has lost the ability to divide, but the cell may still be metabolically active, and such cells must therefore be accounted for in a PBM because they still consume the substrates in the growth media. Alternatively, a cell may be considered dead if it is no longer metabolically active, but until it lyses, it is still present in the culture and will show up in measurements such as microscope or electronic particle counting. These dead but not yet lysed cells may therefore also have to be accounted for in a model. A model can account for these different types of cells by including a population balance equation for each type. The first type, the subpopulation of living, dividing and metabolically active cells, can be modeled by a standard population balance similar to Eq. (2.2):

$$\frac{\partial W_1}{\partial t} + \frac{\partial r_1 W_1}{\partial z} = 2\int_0^\infty \Gamma_1(\tilde{z}) W_1(\tilde{z}, t) p_1(z, \tilde{z})\, d\tilde{z} - \left(D + \Gamma_1(z) + \Theta_1(z)\right) W_1(z, t)$$

where the subscript 1 indicates that the balance and the various functions refer to only this first subpopulation of cells, metabolically active and dividing cells. However, in this balance, the function Θ_1 is the rate at which the cells transition to cells of the second type, cells that are metabolically active but have ceased to divide. It should thus properly be called a *transition inten-sity* and not a *death intensity*. The second subpopulation of cells can be modeled by a slightly modified population balance with a division intensity equal to zero and a source term accounting for the transition of cells from type 1 to type 2,

$$\frac{\partial W_2}{\partial t} + \frac{\partial r_2 W_2}{\partial z} = -\left(D + \Theta_2(z)\right) W_2(z, t) + \Theta_1(z) W_1(z, t)$$

where the function Θ_2 is the rate at which type 2 cells are changed into type 3 cells, cells that neither divide nor are metabolically active. The population balance for this subpopulation is quite simple since both the single-cell growth rate r and the division intensity are identically zero:

$$\frac{\partial W_3}{\partial t} = -\left(D + \Theta_3(z)\right) W_3(z, t) + \Theta_2(z) W_2(z, t)$$

where the function Θ_3 is the rate at which these cells lyse and finally disappear completely from the culture.

These last three equations form a model of a cell culture in which cells die by first losing the ability to divide, then ceasing to be metabolically active and finally by lysing. The model consists of three coupled, unstructured population balances (plus boundary conditions and substrate and product equations). In this model, each cell is characterized by two parameters, the state z and an index, 1, 2 or 3, that identifies the subpopulation to which the cell belongs. The model is thus formally a structured population balance model. The simplifying modeling assumption, that a cell population can be split into separate subpopulations, each of which can then be modeled by an unstructured population balance, is often a convenient trick for modeling complex populations that would otherwise need structured models.

2.2.4 Single-cell growth rate *r*

Of the various functions that appear in a population balance, the single-cell growth rate is the least mysterious. This function models the cell metabolism and describes how fast the state z of a cell changes as a function of z itself and of the substrate concentrations. The last decade has seen an enormous amount of literature that addresses the issue of how to model the metabolism of the cell, and it is only a matter of time before one will be able to formulate acceptable metabolic models based exclusively on the genomic sequence of the organism. Unfortunately, all these models have high dimensionality, involving hundreds of coupled ordinary differential equations, and are therefore practically useless from the standpoint of population balances that are only tractable for very low dimensional state spaces.

Unstructured population balances require unstructured models of the single-cell growth rate, and it is natural to seek inspiration from, and possibly apply, the unstructured models of population growth rates that have been used in unstructured, distributed models. The arguably most famous and frequently used such model is the Monod model [60, 61]. In our nomenclature,

$$r(z, C_S) = \frac{v_m C_S}{K + C_S} z \tag{2.7}$$

where C_S is the substrate concentration, v_m the maximum specific growth rate of the cell, and K a saturation constant, often just called the Monod constant. The Monod model is popular because it offers a good compromise between mathematical simplicity and realism. However, many other growth rate expressions have been used in distributed models, and all of these can be adapted to population balances. For instance, the Blackman model [9] is a piecewise linear approximation of the Monod model:

$$r(z, C_S) = \begin{cases} v_m, & C_S \geq 2K \\ \dfrac{v_m C_S}{2K}, & C_S \leq 2K \end{cases}$$

Being piecewise linear as opposed to nonlinear, the Blackman equation can give PBMs that are easier to solve analytically than the Monod model, but the discontinuity in the slope of r is biologically unreasonable and is a nuisance to deal with. The Moser model [62] is a generalization of the Monod model:

$$r(z, C_S) = \frac{\nu_m C_S^n}{K + C_S^n} z$$

and reduces to the Monod model for $n = 1$.

Instead of using these models, which although biologically reasonable were intended for a population of cells and not single cells, one can formulate simple models of single-cell growth. Bertalanffy [96] pointed out that the growth rate of a cell equals the difference between the rate of substrate uptake and the rate of transformation or release of cell matter into the medium. When nutrient uptake is limited by the cell surface, then this rate can be modeled once the shape of the cell is known, while the rate of release of material can reasonably be modeled as proportional to the cell mass. Thus, the rate of single cell mass growth rate can be modeled

$$r(m, C_S) = k_{\text{uptake}}(C_S)\text{cell surface area} - k_{\text{rel}}\, m$$

where k_{uptake} and k_{rel} are proportionality constants. The uptake rate will generally be a function of the substrate concentration C_S, while there is little reason to assume the release rate will be a function of this. Eakman [27, 28] used this equation to derive growth rate models for cocci (spherical cells):

$$r_{\text{sphere}}(m, C_S) = k_{\text{uptake}}(C_S) \left(\frac{36\,\pi}{\rho^2}\right)^{1/3} m^{2/3} - k_{\text{rel}}\, m$$

where ρ is the density of the cell. And for rods,

$$r_{\text{rod}} = \left(\frac{2k_{\text{uptake}}(C_S)}{R\,\rho} - k_{\text{rel}}\right) m + \frac{4}{3}\,\pi\, R^2 k_{\text{uptake}}(C_S)$$

where R is the radius of the rod, assumed constant. The substrate dependence of the proportionality constant for uptake, k_{uptake}, was modeled by a Monod-type expression

$$k_{\text{uptake}}(C_S) = \frac{k' C_S}{K + C_S}$$

Clearly other models of substrate dependence can be substituted at this point.

Expressions for the single-cell growth rate lead to a differential equation for the cell state as a function of cell age or time:

$$\frac{dz}{dt} = r(z, C_S)$$

The solutions to this equation are the single-cell growth curves. Assuming constant density and substrate concentration, the mass growth curves for spherical and rod-shaped cells are found from the expressions above as [27, 28]

$$m_{\text{sphere}}(t) = \left[\frac{k_{\text{uptake}}}{k_{\text{rel}}} \left(\frac{36\,\pi}{\rho^2} \right)^{1/3} \left(1 - e^{t \cdot k_{\text{rel}}/3} \right) + m_0^{1/3} e^{t \cdot k_{\text{rel}}/3} \right]^3 \quad (2.8)$$

and

$$m_{\text{rod}}(t) = m_0 e^{(2k_{\text{uptake}}/R\rho - k_{\text{rel}}) \cdot t} + \frac{4\pi R^2 k_{\text{uptake}} (e^{(2k_{\text{uptake}}/R\rho - k_{\text{rel}}) \cdot t} - 1)}{3\left(\frac{2k_{\text{uptake}}}{R\rho} - k_{\text{rel}} \right)} \quad (2.9)$$

where m_0 is the initial cell mass. The two families of growth curves are graphed in Figs. 2.4 and 2.5, respectively.

For cocci or spherical cells, the specific surface area of the cell, the surface area per volume, decreases as the cells grow, and the rate of uptake therefore does not increase with cell mass as fast as the rate of release. Consequently, the growth curves approach an asymptote, the upper mass limit of spherical cells, where the rate of substrate uptake equals the rate of release of cell matter. As the asymptote is approached, the growth curves converge and the state space is said to contract.

The growth curves for rods, on the other hand, are approximately exponential functions; they increase without limit and diverge as the cells age. In this situation, the state space is said to expand.

Expansion or contraction of state space has a simple effect on the shape of the distribution of states. Neglecting division and death, it is clear that when the growth curves are straight parallel lines, the state space neither contracts nor expands and the distribution of states simply shifts toward higher values of z with the velocity $r(z)$. When the state space expands, the population of cells within a given interval of cell states will shift toward a wider interval of states. Assuming no cell divisions or death, the number of cells in this population must remain constant and the integral of the distribution of states over this expanding interval must therefore be constant. This is possible only if the value of the distribution of states decreases as the interval expands. Thus, the overall effect of state space expansion is to stretch the distribution of states over a wider range of cell states while simultaneously

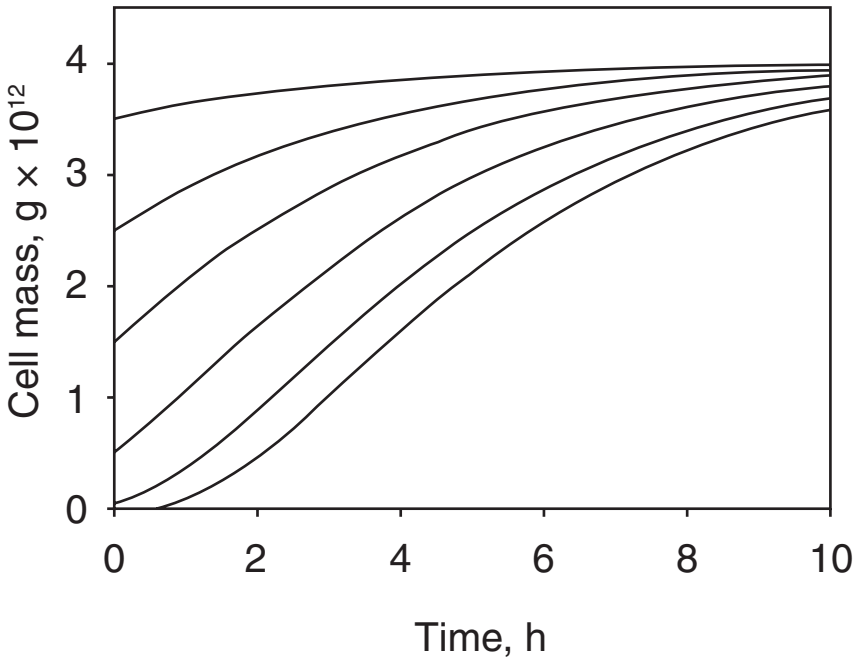

Figure 2.4 A family of cell mass growth curves for spherical cells or cocci, Eq. (2.8). Parameter values are taken from [27] and are as follows: $\rho = 1.01$ g/cm^3, $k_{\text{uptake}} = 3.306 \cdot 10^{-5}$ g/(cm$^2 \cdot$ h), $k_{\text{rel}} = 1$ h^{-1}.

lowering its value such that the area under the distribution remains constant. This effect takes place while the distribution shifts toward higher values of the state parameter z with a velocity equal to the single-cell growth rate; see Fig. 2.6 left. Similarly, the overall effect of state space contraction is to make the distribution of states more narrow while increasing its value and shifting it toward higher values of z; see Fig. 2.6 right.

An illustration of this effect is seen in traffic flow: Traffic flow can be modeled by a population balance on the cars, in which the state parameter is the position of the car. In this case, the velocity of a car takes the place of the growth rate of a cell and the distribution of states is simply the density of the cars on a stretch of road. The state space expands when the cars accelerate, for instance, when they enter a freeway, and the density of cars must therefore decrease. As everyone knows, this is in fact what is observed: The density of cars does decrease, or equivalently the distance between cars increases, as the speed of the cars increases. Conversely, when traffic is forced to slow down, for instance when passing road repair, the density goes up; the cars are closer together.

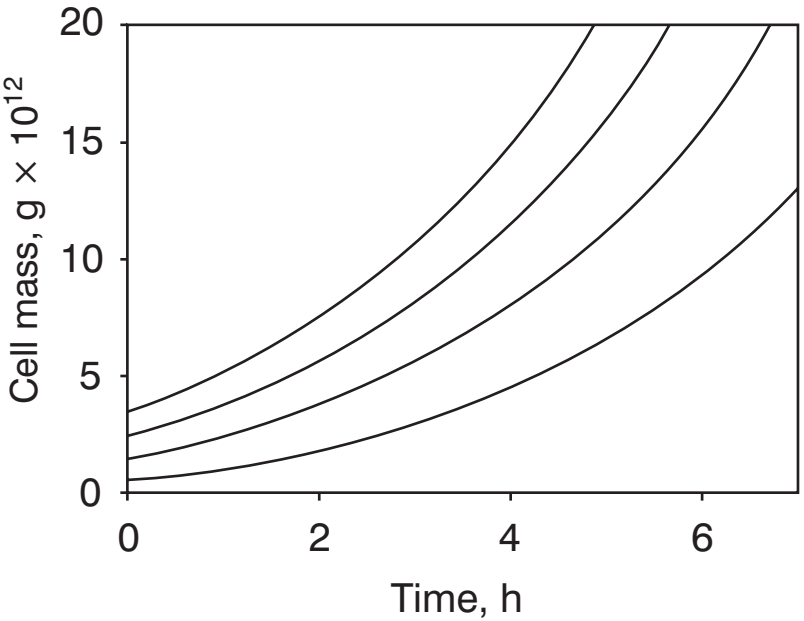

Figure 2.5 A family of cell mass growth curves for rods, Eq. (2.9). Parameter values are taken from [27] and are as follows: $\rho = 1.01$ g/cm^3, $k_{\text{uptake}} = 3.306 \cdot 10^{-5}$ g/(cm$^2 \cdot$ h), $k_{\text{rel}} = 1$ h^{-1}, $R = 5 \cdot 10^{-5}$ cm.

2.3 Some Properties of PBMs

A couple of points are worth making regarding the properties of Eq. (2.2). Recall that $N(t)$, the cell number concentration, equals the zeroth moment of the distribution of states. Thus, by taking the zeroth moment of the population balance equation itself, one obtains an equation for $N(t)$ versus time:

$$\begin{aligned}\frac{dN}{dt} &+ r(\infty)W(\infty, t) - r(0)W(0, t) \\ &= 2\int_0^\infty \Gamma(\tilde{z})W(\tilde{z}, t)\int_0^\infty p(z, \tilde{z})dz\,d\tilde{z} \\ &- DN(t) - \int_0^\infty (\Gamma(z) + \Theta(z))W(z, t)dz\end{aligned}$$

Using the regularity conditions, Eqs. (2.3) and (2.4), respectively, the last two terms on the left-hand side equal zero. Using Eq. (2.6) to get rid of the integral of $p(z, \tilde{z})$, we then get

$$\frac{dN}{dt} = -DN(t) + \mu(t)N(t)$$

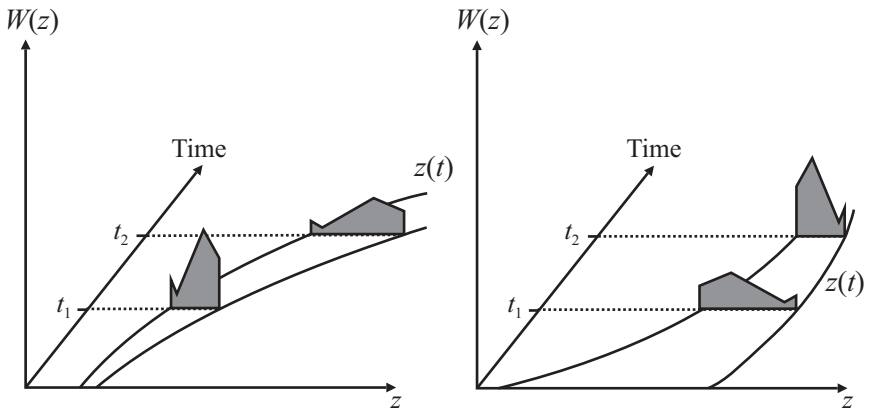

Figure 2.6 The effect of state space expansion (left) is to increase the width of the distribution of states while simultaneously lowering its value and shifting it toward higher values of the cell state parameter, z. Contraction (right) has the opposite effect of making the distribution more narrow while increasing its value.

where μ is the specific growth rate of the cell population, found as

$$\mu(t) = \int_0^\infty (\Gamma(z) - \Theta(z)) \; f(z, t) dz \tag{2.10}$$

It probably cannot be mentioned too often that PBMs are models that associate the kinetics of the entire cell population, represented here by $\mu(t)$, the specific growth rate in cell number, with the kinetics of the single cell. Equation (2.10) is a key relationship in this connection and can often be simplified once $f(z, t)$ is known. Equations of this type, linking population kinetic parameters, such as μ, to single-cell kinetic parameters, will be called *linkage equations*. Note that at steady state $D = \mu$, and the steady-state dilution rate will therefore often be used to characterize population growth in lieu of the specific growth rate.

Another useful relationship is obtained by calculating the first moment of the steady-state version of Eq. (2.2). One obtains after simplifications

$$\int_0^\infty r(z)W(z)dz = D\int_0^\infty zW(z)dz + \int_0^\infty z\Theta(z)W(z)dz \tag{2.11}$$

and in the special case where there is no death and single cell kinetics is first order, $r(z) = \nu z$, this reduces to

$$D = \nu \quad \text{or equivalently} \quad \mu = \nu$$

showing that, under the assumption of no death and first-order single-cell kinetics, the single-cell specific growth rate equals the population specific growth rate.

The specific growth rate of the population μ is generally a function of the substrate concentration, as modeled by, for instance, Monod's model, Eq. (2.7). In Eq. (2.10), this dependence is of course manifested through the distribution f's dependence on the substrate, but also through the dependence of Γ and Θ on substrate concentration. However, even under conditions of constant substrate concentration, the specific growth rate of the population is constant only if the distribution of states is not a function of time. For instance, a culture can be inoculated with a population of very young cells, with a narrow distribution of states situated at low values of the state space parameter. The cells in this culture must pass through several cell cycles before the initial cell cycle synchrony is lost and before the distribution of states attains its steady-state shape. During this period, the population specific growth rate calculated from Eq. (2.10) will change with time, without this being in any way caused by changes in the substrate concentrations. This time dependence of μ, caused by transients in the distribution of states, is a phenomenon that can be captured only by population balance models, never by distributed models.

A frequently encountered concept in cell growth is the simplifying notion of exponential growth. This is used to describe steady-state growth in a chemostat or growth in a batch culture under conditions when substrate limitations are not significant. Under such conditions, both $\Gamma(z)$ and $\Theta(z)$ are independent of time and it is reasonable to seek an asymptotic solution to the PBE, valid when the distribution of states has reached a steady shape. This solution will have the form

$$W(t, z) = f(z)e^{\mu t}$$

where μ is the specific growth rate of the population. Substituting this expression into the population balance for a batch reactor ($D = 0$) gives, after simplifications,

$$\frac{dr(z)f(z)}{dz} = \int_0^\infty \Gamma(\tilde{z})f(\tilde{z})p(z, \tilde{z})d\tilde{z} - (\Gamma(z) + \Theta(z) + \mu)f(z)$$

This is the equation for the steady-state distribution in a chemostat with dilution rate $D = \mu$, and the two situations are therefore mathematically equivalent. The result states that the kinetics in a steady-state chemostat is analogous to that of exponential batch

growth, assuming that the shape of the distribution of states does not change with time.

2.4 Substrate and Product Balances

The equation for substrate or product concentration is obtained, as for unstructured distributed models, by introducing some appropriate yield Y, defined as the rate of biomass formation over the rate of substrate consumption. However, the yield can be different for different cell states, and the total rate of substrate production or product formation must be found by integrating the rate of consumption/production by cells in a specified state over all possible cell states. Thus, for a chemostat one obtains

$$\frac{dC_S}{dt} = D(C_{Sf} - C_S) - \int_0^\infty \frac{r(z)}{Y(z)} W(z, t)\, dz$$

where C_S is the substrate concentration in the chemostat and in the exit stream, C_{Sf} the substrate concentration in the feed stream, and $Y(z)$ is the yield of cells in state z. Similarly, the product balance takes the form

$$\frac{dC_P}{dt} = D(C_{Pf} - C_P) + \int_0^\infty r_P(z) W(z, t)\, dz$$

where C_P and C_{Pf} are the product concentrations in the chemostat and in the feed respectively, and $r_P(z)$ is the rate of product formation in cells in state z.

2.5 The Age Distribution

Cell age is not conserved in a division, and the age population balance therefore does not have the same structure as the population balance in Eq. (2.2). Like any population balance, the age distribution population balance can be derived from a cell balance on a macroscopic control volume as was done for Eq. (1.1), or on a differential control volume like Eq. (2.2). A derivation using probabilistic arguments will be used below to derive the age population balance to emphasize the point that the distribution of states is a statistical concept; it represents the probability that a cell chosen at random is in a specified state. For more detailed steps of the derivations, see Refs. 33 and 94.

Consider a culture in a chemostat at steady state. All the events that can possibly occur to a cell of age a between the time t and $t + dt$ are

- E_{age}: The cell remains in the chemostat and attains the age $a + da$.
- E_{washout}: The cell washes out of the chemostat.
- E_{death}: The cell dies.
- E_{division}: The cell divides.

Keep in mind that these probabilities are for cells of age a that are present at time t and in that sense are conditional probabilities. Since the events are mutually exclusive, their probabilities $P(E)$ must sum to 1:

$$P(E_{\text{age}}) + P(E_{\text{washout}}) + P(E_{\text{death}}) + P(E_{\text{division}}) = 1$$

or

$$P(E_{\text{age}}) = 1 - P(E_{\text{washout}}) - P(E_{\text{death}}) - P(E_{\text{division}})$$

The probabilities on the right-hand side can all be written as

$$P(E_{\text{washout}}) = -Ddt$$

$$P(E_{\text{death}}) = \Theta(a)dt$$

where $\Theta(a)$ is the probability that a cell of age a will die in the next dt time interval. This is, of course, the death intensity, but defined slightly differently than in Eq. (2.1), where it was defined in terms of the fraction of cells that die. The difference in definition is a matter of interpretation rather than substance, because the final form of the population balance is the same whether the balance is derived from probabilistic arguments or not. Similar comments can be made regarding the division intensity $\Gamma(a)$. In the probabilistic context, it will be interpreted as the probability that a cell of age a divides in the next dt time interval. Thus,

$$P(E_{\text{division}}) = \Gamma(a)dt$$

and

$$P(E_{\text{age}}) = 1 - (D + \Theta(a) + \Gamma(a))dt$$

The number of cells that have age $a - da$ at time t is given by the age distribution as $W(a - da, t)da$. The number of these cells that remain in the chemostat as cells of age a at time $t + dt$, i.e., the cells that do not wash out, die or divide, is $W(a - da, t)da$ multiplied by the probability of the cell not washing out, dying, or dividing, i.e.,

$$\text{Number of cells of age } a \text{ at time } t + dt = W(a, t + dt)$$
$$= W(a - da, t)da(1 - (D + \Theta(a - da) + \Gamma(a - da))dt)$$

Subtracting from this the number of cells with age a that were present in the chemostat at time t, i.e., $W(a,t)da$, gives, after rearrangement,

$$\frac{W(a, t + dt) - W(a, t)}{dt} + \frac{W(a, t) - W(a - da, t)}{dt}$$
$$= -(D + \Theta(a - da) + \Gamma(a - da))W(a - da, t)$$

Evidently, $da = dt$, so this becomes

$$\frac{\partial W}{\partial t} + \frac{\partial W}{\partial a} = -(D + \Theta(a) + \Gamma(a))W(a, t) \tag{2.12}$$

A cell number balance on cells of zero age leads to a boundary condition of the form

$$W(0, t) = 2\int_0^\infty \Gamma(a)W(a, t)\ da \tag{2.13}$$

The factor of 2 results from the fact that cell divisions produce two new cells of age zero. This equation is commonly referred to as the *renewal equation*.

2.5.1 Age division intensity

The age population balance has only one breakage function, the division intensity $\Gamma(a)$. This division intensity can in principle be obtained from mechanistic models of the cell cycle.

If the cell cycle is modeled as a completely deterministic process, then all divisions occur at the boundary of state space, and determining the division intensity is equivalent to determining this boundary. However, specifying the boundary may well require an exceedingly detailed description of the cell state and a more reasonable modeling approach is probably to use stochastic models. Stochastic effects can enter models on two grounds. Many of the regulatory proteins that control progress through the cell cycle are present in low amounts, and the usual assumption in chemical kinetics of continuity of concentration may not be valid. Secondly, any tractable cell cycle model must include some amount of lumping, and the state at cell division can therefore not be specified sufficiently accurately to determine the boundary of the state space.

A general approach to stochastic chemical kinetics has been presented by Gillespie [35] who defines the so-called reaction probability density function as

$$P(t, \tau, n) = \text{probability that at time } t \text{ the next reaction will be of type } n \text{ and occur at time } t + \tau$$

which is simply a probability density function and, in general, a complicated function of the number of molecules of each kind in the reacting system. However, in the simple cell cycle models that will be considered below, we will assume that all the reactions in the model are elemental first-order reactions of the type $X_{n-1} \rightarrow X_n$, for which the reaction probability density function can be shown to be

$$P(t) = Ce^{-Ct} \tag{2.14}$$

where C is a constant defined such that $C\,dt$ equals the probability of the reaction $X_{n-1} \rightarrow X_n$ occurring in the next dt time interval.

A cartoon model of the cell cycle [50] assumes that progress through the cell cycle can be modeled as progress through a set of reactions in series, say conversion of an initial reactant X_1, through the intermediates X_n to the final product X_N. The reaction probability density function for the overall reaction from X_1 to X_N can be found in terms of the reaction probability density functions for each of the individual reactions. The reaction probability density function for the reaction $X_n \rightarrow X_N$ will be indicated $P_{n \rightarrow N}(t)$ and is defined as

$$P_{n \rightarrow N}(t)dt \equiv \text{probability that the next } X_n \rightarrow X_N \text{ reaction will occur in the differential time interval } (t, t + dt)$$

where we have assumed that the current time equals zero. Clearly, the reaction $X_1 \rightarrow X_N$ will only occur at the time t if the reaction $X_1 \rightarrow X_n$ occurs at a time $\tau < t$ and is followed by the reaction $X_n \rightarrow X_N$ after a time $t - \tau$. Thus, the reaction probability density function $P_{1 \rightarrow N}$ must be the product of these two reaction probabilities, integrated over all possible values of τ:

$$P_{1 \rightarrow N}(t) = \int_0^t P_{1 \rightarrow n}(\tau) P_{n \rightarrow N}(t - \tau)\, d\tau$$

which is a well-known result for the distribution of the sum of two stochastic variables. The result can be used recursively to derive models of the reaction probability density function for a reaction sequence in terms of the reaction probability density functions of simpler reactions.

Consider the case where each reaction step can be modeled as a fundamental reaction for which the reaction probability density function is given by Eq. (2.14). It is easily shown by induction that the reaction probability density function for N steps is

$$P_N(t) = C\frac{(Ct)^{N-1}}{(N-1)!}e^{-Ct}$$

which is the Erlang distribution. This distribution represents the probability that a newborn cell, picked at random, will divide after a time period t, i.e., it is the a priori distribution of division ages. The division intensity can be found from the a priori distribution of division ages as [27]

$$\Gamma_s(a) = \frac{P_N(a)}{1 - \int_0^a P_N(x)\ dx} = \frac{C}{(N-1)!}\frac{(Ca)^{N-1}}{\sum_{n=0}^{N-1}\frac{(Ca)^n}{n!}}$$

The a priori distribution of division ages and the division intensity for the reaction in series model are plotted in Fig. 2.7.

Another cartoon model of the cell cycle assumes that division occurs after some number of parallel reactions have occurred [72]. Consider the case of two parallel reactions and assume for simplicity that both have the same exponential reaction probability density function, Ce^{-Ct}. The reaction probability density function at t for the two parallel reactions is then the probability that the first reaction occurs precisely at t while the other reaction has already occurred plus the probability that the second reaction occurs precisely at t while the first reaction has already occurred. Thus,

$$P(t) = \int_0^t P_1(\tau)\ d\tau P_2(t) + \int_0^t P_2(\tau)\ d\tau P_1(t) = 2Ce^{-Ct}(1 - e^{-Ct})$$

Replacing t with cell age we obtain the a priori distribution of division age for this model. The division intensity becomes

$$\Gamma(a) = \frac{2Ce^{-Ca}(1 - e^{-Ca})}{1 - \int_0^a 2Ce^{-C\tau}(1 - e^{-C\tau})d\tau} = 2Ce^{-Ca}\frac{1 - e^{-Ca}}{2e^{-Ca} - (e^{-Ca})^2}$$

This result is easily generalized to N identical reactions in parallel. The a priori distribution of division ages is

$$P_N(a) = NCe^{-Ca}\left[\int_0^a Ce^{-Ct}dt\right]^{N-1} = NCe^{-Ca}(1 - e^{-Ca})^{N-1}$$

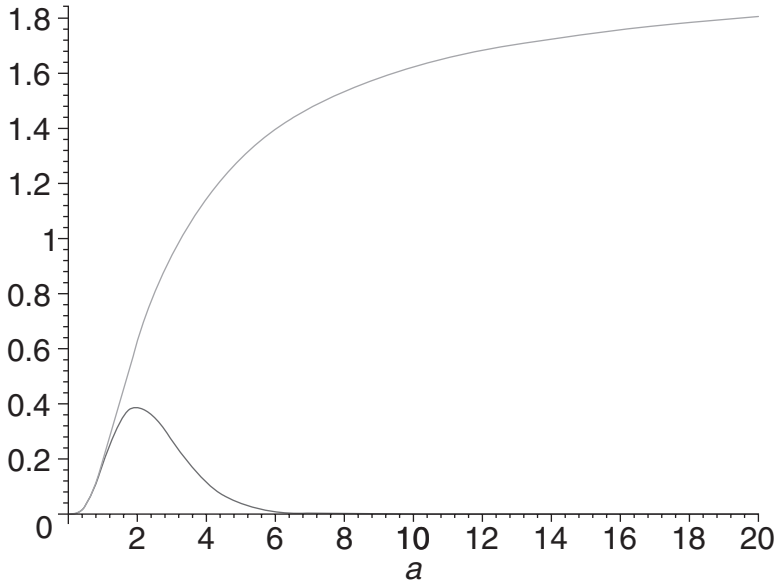

Figure 2.7 The a priori distribution of division ages and the division intensity versus age for the series reaction model with $C = 2$ and $N = 5$.

and the division intensity becomes

$$\Gamma_p(a) = N\ C\ e^{-Ca}\frac{(1-e^{-Ca})^{N-1}}{1-(1-e^{-Ca})^N}$$

The a priori distribution of division ages and the division intensity for the parallel reactions model are plotted in Fig. 2.8.

The division intensity is usually assumed to go to infinity as the value of the argument increases, but for the two models considered here, this is not the case; $\lim_{a\to\infty}\Gamma_s(a) = \lim_{a\to\infty}\Gamma_p(a) = C$. This finite limit for the age division intensity may actually be a better reflection of reality than models that use unbounded division intensities. If a cell has undergone almost all reactions required for division and is waiting for the final reaction to occur, aging by itself does not increase the probability of this reaction occurring and the division intensity must therefore be constant. It is similar to standing at the curb and waiting to catch a taxi. You are continuously getting older but your aging by itself does not increase the chance of a taxi coming by.

Both of the above models of the age division intensity can be described as transition probability models. These kinds of models have appeared in several studies [13, 14, 84, 86, 87] but have also been severely criticized [52]. Their basic problem is that they assume that cell age is

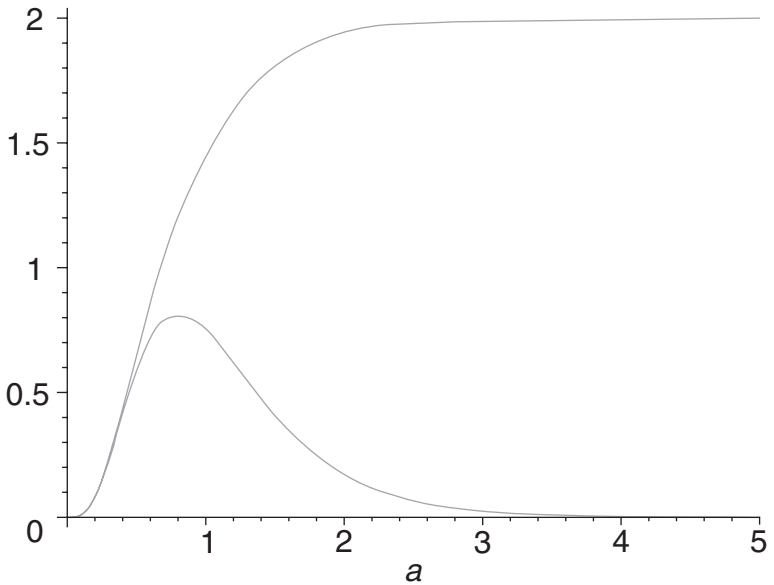

Figure 2.8 The a priori distribution of division ages and the division intensity versus age for the parallel reactions model with $C = 2$ and $N = 5$.

sufficient to characterize the cell's state and the probability of cell division. However, this assumption quickly leads to problems because newborn cells are bound to exhibit some size and mass differences and, if cell age is all that determines whether a cell divides or not, this distribution of sizes of newborn cells will broaden with each generation, preventing the formation of a steady-state distribution of cell sizes. A rigorous version of this handwaving argument has been presented for exponential growth [5]. The conclusion is obviously nonsense and progress through the cell cycle must therefore depend on other cell state parameters such as size or mass. However, this requires a structured population balance model which is not the subject of this chapter.

2.6 Problems

2.1 Derive the population balance equation, including the substrate and product equation for a fed-batch reactor with volumetric feed rate $Q(t)$. Take the zeroth moment of the PBE and obtain the distributed model for this case.

2.2 Derive Eq. (2.11).

Chapter

3

Steady-State Solutions

Even steady-state population balance models are so mathematically complex that analytical solutions are possible only in special cases. However, it is possible to obtain models that are mathematically quite simple by making use of the concept of cell cycle control points. Although crude, control point models can be solved analytically and the solutions do provide valuable insight into the properties of the distribution of states and how it changes with growth conditions and with different kinds of cell cycle controls. Working with these simple control point models also builds one's insight and intuition about population balances and their solutions, intuition that can be extremely valuable when progressing to analysis of more realistic models. This chapter first covers control point models in some depth before describing the special cases in which analytical solutions can be obtained without the assumption of control points.

3.1 Control Points

Living cells differ from almost all other types of particles in that growth and division processes are tightly controlled by groups of regulatory proteins usually known as the *cell cycle control system* [2]. The details of the control system vary from organism to organism, but all must direct the replication of the genome prior to cell division and the partitioning of the two genome copies as well as other essential cell components and organelles between the two daughter cells formed in the division. The cell cycle control system is similar in all eukaryotes and is customarily divided into a sequence of consecutive phases, the G1, S, G2, and M phases. The terms stand for first gap (G1), the period

following division during which DNA replication has not yet begun; synthesis (S), the period during which the genome is duplicated; second gap (G2); and mitosis, or cell division (M). The length of the G1 phase in particular varies with growth conditions, being brief during optimal growth conditions and long during poor growth conditions. Control of the duration of the G1 phase is thus the primary way many eukaryotic cells control the duration of the cell cycle and therefore the growth rate of the entire population of cells.

Several checkpoints have been identified in the cell cycle, points at which progress through the cycle will cease if the cell cycle control system detects a deviation from normal. For instance, the G1 and G2 phases contain DNA damage checkpoints in which the cell cycle arrests while any damage to the DNA is repaired. Likewise, there are checkpoints that stop the cell from entering mitosis if the DNA has not been replicated and halt chromosome partitioning until all chromosomes are attached to the mitotic spindle fibers.

The idea of cell cycle checkpoints can be used as a modeling concept to formulate simple population balance equations, by assuming that cell divisions and births occur only at discrete points in the cell cycle, points that will be called *control points* to distinguish the modeling concept from the biological concept of a cell cycle checkpoint [42]. The control points partition the state space into disjoint intervals, and in these intervals the division intensity and the distribution of birth states is therefore identically zero. Thus, in each such interval, the steady-state population balance equation is reduced to an ordinary first-order differential equation, which is readily solved:

$$\frac{d}{dz}(r(z)W(z)) = -DW(z)$$

The division and birth processes are then accounted for through cell balances over the control points. To illustrate how these balances are obtained, consider a binary fission organism that divides when the cell mass M has been attained. New cells must necessarily form at the mass $M/2$. We now consider a control volume, split into two parts, with one part being the point M and the other the point $M/2$; see Fig 3.1. Notice that this control volume composed of points has zero "physical" volume.

The cells in the flux growing into the control volume at M all exit the control volume by division. Division of these cells gives rise to twice as many new cells, and all the new cells that are born at $M/2$ leave by growth. The cell balance therefore states that the growth flux out at $M/2$ equals twice the growth flux in at M, or

$$2r(M)W(M) = r(M/2)W(M/2)$$

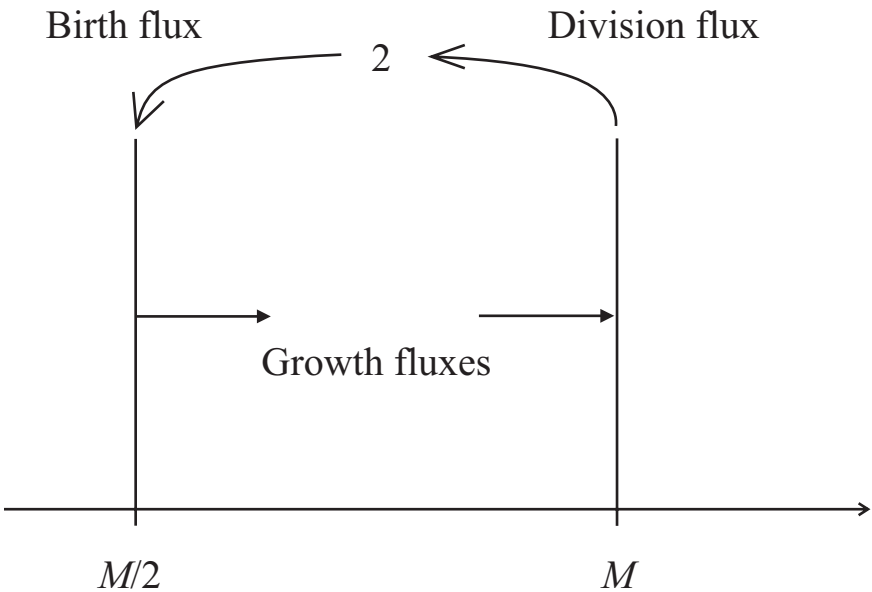

Figure 3.1 Cell balances around division and birth points for binary fission.

Models can use cell balances that are more complex than shown in this example. For instance, one can model cell division as occurring at several division control points with some given fraction of the cells dividing at each point, and division need not be modeled as binary but can assume any number of discrete birth points. When setting up a cell balance on any control point, one equates the flux into the control point and the flux out of the control point. A point has no volume and therefore there is no accumulation term in a control volume balance. If the control point is a point of cell birth, then the cell balance states that the growth flux into the point plus the sum of the birth fluxes into the point equals the growth flux out of the point. This is conceptually similar to a mixing point in a classical chemical engineering flow system with several streams entering the point and one stream leaving. Similarly, a control point for division states that the growth flux into the point equals the sum of the growth and division fluxes out of the point. This is conceptually similar to a splitting point in a classical chemical engineering flow system. A division flux is equal to the growth flux into the control point multiplied by the fraction of cells that divide at this point and each division flux gives rise to a birth flux twice as large as the division flux. However, this birth flux may be split among several birth control points. Thus, all fluxes which appear in cell balances over control points are proportional to growth fluxes which have the form $r(z)W(z)$, and cell

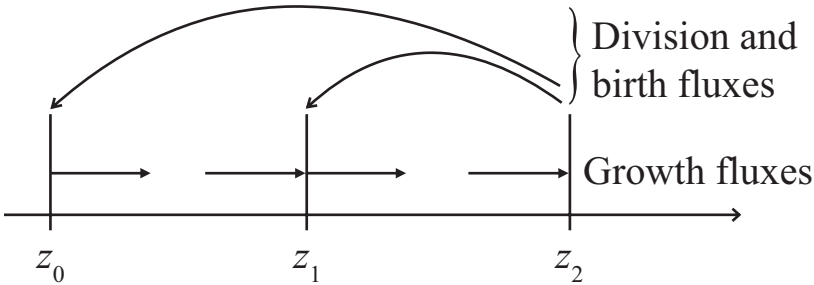

Figure 3.2 Cell fluxes when cell division results in two cells in different states.

balances over control points are therefore linear combinations of terms of this form.

Consider for instance the case shown in Fig 3.2, a cell cycle where all cells divide when they reach the state z_2 and the newborn cells have the states z_0 and z_1 respectively. If z is conserved in a division then $z_2 = z_0 + z_1$, but we need not make that assumption here. Without loss of generality, we can assume that the state z_1 represents cells that are older than cells in state z_0.

A cell balance over the control point z_0 states that the growth flux out equals the birth flux in. But the birth flux in equals twice times half (half, since only half of the cells formed in a division go to z_0) of the division flux out of the control point z_2, and the division flux out at z_2 equals the growth flux in at z_2. Considering a control volume made up of the control points at z_0 and z_2, the cell balance becomes

$$\tfrac{1}{2} \cdot 2 \cdot r(z_2)W(z_2) = r(z_0)W(z_0)$$

The control point at z_1 is similar to a mixing point in which the flux out from z_1 equals the sum of the growth and the birth fluxes into the point. The birth flux into the point is identical to twice times half the division flux in the previous cell balance so we get

$$\tfrac{1}{2} \cdot 2 \cdot r(z_2)W(z_2) + r(z_1)W_-(z_1) = r(z_1)W_+(z_1)$$

where the subscripts – and + indicate the limit of $W(z)$ when the free variable approaches the argument through lesser or greater values. We have to specify that $W(z)$ is calculated this way because the function is discontinuous at the control point. This notation is inconvenient, and it is better to do the following: The control points partition the state space into discrete intervals in which no divisions or births occur so in each of these intervals, the distribution of states must be continuous. Number these intervals consecutively and denote the distribution of states in the nth interval $W_n(z)$ or $f_n(z)$. The cell balances can then be

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written as linear combinations of terms of the form $r(z)\ W_n(z)$. For the case considered above, the first interval runs from z_0 to z_1 and the second interval from z_1 to z_2 so the cell balances take the form

$$r(z_2)W_2(z_2) = r(z_0)W_1(z_0)$$
$$r(z_2)W_2(z_2) + r(z_1)W_1(z_1) = r(z_1)W_2(z_1)$$

Of course, each W_n will be governed by its own PBE, which, when solved, will generate an arbitrary constant. As the number of intervals goes up, so does the number of cell balances, and if the model is formulated correctly the number of cell balances will equal the number of PBEs, or equivalently, the number of arbitrary constants. However, it turns out that even though the number of cell balances equals the number of arbitrary constants, it is not possible to solve for all the arbitrary constants. One constant will always remain unknown. It must necessarily be so because both the PBEs and the cell balances are homogeneous and can be multiplied through by an arbitrary constant to obtain an identical set of equations. The solution to the PBE is therefore determined only up to a constant multiplier. Specifically, one can divide through by the cell number concentration N and obtain balances for the normalized distribution $f(z)$. In the language of linear algebra: the equations between the arbitrary constants, the equations obtained when the solutions to the PBEs are substituted into the cell balances, are linear and homogenous and therefore have an infinity of nonzero solutions, solutions in which all but one of the arbitrary constants is found in terms of this constant. This remaining arbitrary constant that cannot be found from the cell balances must generally be found by using a substrate balance.

One cannot conclude from the comments above that one of the cell balances is superfluous; this is not the case. All the balances are needed because, when solved, they yield not only the arbitrary constants minus one, but also a linkage equation similar to Eq. (2.10), which relates the parameters of single-cell growth kinetics, such as the single-cell mass growth rate, to the parameters of the population growth, such as the specific growth rate μ. This equation is an important part of the solution to a control point PBM and should always be reported.

Cell balances over control points at steady states are especially simple because the control points are fixed in time. However, the point at which they are fixed may depend on various system parameters such as the concentrations of substrates, but this dependence simply adds additional algebraic equations to the problem, it does not affect the way the balances themselves are written. In the general transient case, the states at which cells divide and are born may change with time. This

complicates calculation of the fluxes into and out of the control points and will be considered later in the section on transient solutions.

The examples that follow should make all these ideas clearer.

Example 3.1: Cells with a single division age For cells that all divide at the age a_d the steady-state PBE is

$$\frac{df}{da} = -Df \Rightarrow f(a) = Ce^{-Da}$$

Using the normalization condition determines the arbitrary constant *C:*

$$1 = \int_0^{a_d} Ce^{-Da} da \Rightarrow C = \frac{D}{1 - e^{-Da_d}}$$

The cell balance around birth and division is

$$f(0) = 2f(a_d)$$

and when the result for $f(a)$ is substituted in, one obtains

$$1 = 2e^{P - Da_d} \Rightarrow Da_d = \ln(2)$$

This is the linkage equation for this organism, relating the specific growth rate of the population, as given by D, to the single-cell kinetic parameter a_d. The equation is so simple because a_d is the only parameter needed to characterize single-cell kinetics. It is well known that in an exponentially growing population, the doubling time t_d and the specific growth rate are related by a similar equation, $Dt_d = \ln(2)$. The linkage equation therefore shows that the doubling time of a population is equal to the length of the cell cycle, assuming that the length of the cell cycle is the same for all cells in the population.

Example 3.2: Zeroth-order single-cell kinetics Consider an example where cells divide at the mass M, are born at the mass $M/2$, and the single-cell growth rate follows zeroth-order kinetics, i.e.,

$$r(m) = k$$

The control points are at $M/2$ and M, and the distribution of states is identically equal to zero outside this interval. The PBE can now be solved for the normalized distribution of states

$$\frac{dk\,f}{dm} = -Df \Rightarrow f(m) = Ce^{-(D/k)m}, \quad \frac{M}{2} < m < M$$

The normalization condition gives

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$$\int_{M/2}^{M} Ce^{-(D/k)m}\,dm = 1 \Rightarrow f(m) = \frac{De^{-(D/k)m}}{k(e^{-DM/2k} - e^{-DM/k})}$$

The cell balance can now be used to derive the linkage equation between k, the single-cell kinetics parameter, and D

$$2k\,f(M) = k\,f(M/2) \Rightarrow \frac{DM}{k} = \ln(4)$$

Keep in mind that the single-cell kinetics and the population kinetics are different concepts and that it is completely wrong to assume that the specific growth rate of the population equals the specific growth rate of single cells. In fact, as this example shows, the rate equations for the population and for the single cell need not even be the same: Here single-cell growths follow zeroth-order kinetics while population growth follows first-order kinetics.

The linkage equation can be used to simplify the expression for the distribution of states. In our case, one finds that

$$f(m) = \frac{\ln(256)}{M} e^{-\ln(4)m/M}$$

We see that if M is a constant, independent of, e.g., concentrations of components in the medium, then this model predicts a distribution of cell masses that is independent of dilution rate. However, for most organisms, cell size, and thus M, is a function of substrate concentrations, and the distribution of states will therefore change with dilution rate. In order to model this, we will have to introduce an equation for M as a function of the substrate concentration C_S and find the substrate concentration from the substrate balance. Finding the substrate concentration, and with that the cell number concentration, will be the first task. We will assume a single growth limiting substrate with concentration C_S and a constant single-cell yield Y. The consumption term in the substrate balance must be found by integrating the rate of single-cell substrate consumption over all cell states,

$$D(C_{Sf} - C_S) = \int_0^\infty \frac{r(m)}{Y} W(m)\,dm = \frac{N}{Y}\int_0^\infty k\,f(m)\,dm$$

where C_{Sf} is the inlet substrate concentration. For our model, this balance reduces to

$$Nk = YD(C_{Sf} - C_S)$$

However, the problem is still not completely solved. We want to be able to solve for N and C_S as functions of the D and C_{Sf}, the operating parameters of the chemostat, so we need one more equation. The missing equation is a model of how the single-cell growth kinetics depends on the concentration of the limiting substrate. For instance, analogy with the Monod model would suggest

$$k(C_S) = \frac{k_m C_S}{K + C_S} \Rightarrow C_S = \frac{kK}{k_m - k} = \frac{KDM}{\ln(4)k_m - DM}$$

This is now the appropriate time to make use of an equation for $M(C_S)$. It is not possible to write an equation that can serve as a good general model for this dependence; the dynamics of cell cycle control found in nature is simply too rich to be encompassed by a single, simple model. For our case, we will assume that cell size is an increasing function of substrate concentration and use the simplest possible expression for this, a linear dependence

$$M(C_S) = M_0 + \alpha C_S$$

which is substituted into the expression for C_S found just above. Solving for C_S gives the rather unpleasant expression

$$C_S = \frac{\ln(4)\ k_m - D(M_0 + K\alpha) + \sqrt{A}}{2D\alpha}$$

where

$$A = \ln^2(4)\ k_m^2 - \ln(16)\ k_m D(M_0 + K\alpha) + D^2(M_0 - 2\alpha K)^2$$

We can solve for cell number concentration also:

$$N = \frac{Y\ \ln(4)}{M_0 + \alpha C_S}(C_{Sf} - C_S)$$

where we have abstained from substituting in the expression for the substrate concentration. Other quantities of interest can be found, such as, e.g., the washout dilution rate. This is defined as the dilution rate at which the biomass or cell number concentration becomes zero. From the equation above, this occurs when $C_S - C_{Sf}$:

$$D_{\text{washout}} = \frac{S_f k_m\ \ln(4)}{(M_0 + \alpha C_S)(K + C_{Sf})}$$

where, once more, we have left the final manipulation steps to the reader. The biomass concentration X, which is the first moment of $W(m)$, or the average cell mass times the cell number concentration, is

$$X = \int_0^\infty mW(m)\ dm = N\int_{M/2}^{M} m f(m)\ dm = \frac{N(M_0 + \alpha C_S)}{\ln(4)}$$

At this point it is instructive to look back at the solution and try to organize all the information and steps taken to find the solution. Start by noting that

Steady-State Solutions

the complete, well-posed PBM consists of several equations: (1) the PBE and its cell balances,

$$\frac{df}{dm} = -\frac{D}{k} f, \quad 2f(M) = f(M/2)$$

(2) the substrate balance,

$$D(C_{Sf} - C_S) = \frac{N}{Y}\int_0^\infty k\, f(m)\ \ dm$$

and (3) any number of equations that couple the values of the growth parameters to the substrate concentration. In this case,

$$k(C_S) = \frac{k_m C_S}{K + C_S}, \quad M(C_S) = M_0 + \alpha C_S$$

In the general case, the values of the growth parameters may depend on the concentrations of several substrates and products and any number of substrate/product balances may therefore be needed for a well-posed PBM.

Solving the problem means finding all the primary unknowns, the distribution of states, the cell number concentration, and the substrate concentration(s), in terms of the model parameters and the two operating parameters of the chemostat, dilution rate D and substrate feed concentration C_{Sf}.

In solving, it is often slightly more convenient to work with the normalized distribution $f(m)$ instead of $W(m)$ because, using the normalization condition, one can find a solution for f that does not contain the unknown cell number concentration, N.

Start by solving for f. Eliminate the constant of the integration using the normalization condition and substitute the result into the cell balance. What results is the linkage equation between the dilution rate D and the single-cell growth parameters k and M, $DM = k \ln(4)$. This equation is not only an essential part of the result; it often also comes in handy for simplifying the final and intermediate results.

The single-cell growth parameters in this equation are dependent on the substrate concentration, and when these dependencies are substituted in, one obtains an equation from which the substrate concentration can be found as a function of the dilution rate. This solution can be quite complicated in appearance and, in some cases, one may only be able to obtain the solution numerically.

Finally, use the substrate concentration and the solution for f to find the cell number concentration N in terms of the substrate concentration C_S. Because the expression for $C_S(D)$ is often so complicated in appearance, it is a good idea to refrain from substituting it into the expressions for the other results and simply report these in terms of D, C_{Sf}, and C_S, as was done in the solution above for N, X, and D_{washout}.

Example 3.3: Unequal division Find the normalized steady-state distribution of cell mass as a function of dilution rate if cell division results in formation of one cell with mass m_0 and another cell with mass $m_1 > m_0$ and if single-cell mass growth rate follows first-order kinetics with specific growth rate ν.

In this case, the control points of the cell cycle split the state space into two intervals, and we will define $f_1(m)$ as the part of the normalized distribution that lies between m_0 and m_1, and $f_2(m)$ as the part between m_1 and $m_0 + m_1$. For both parts, the PBE takes the form

$$\frac{\nu\, m f}{dm} = -Df(m) \Rightarrow \frac{df}{dm} = -\frac{D+\nu}{\nu}\frac{1}{m}f(m)$$

where ν is the specific single-cell mass growth rate and D is the dilution rate. The solutions to these equations are

$$f_1(m) = \left(\frac{C_1}{m}\right)^{(D+\nu)/\nu}, \quad m \in]m_0,\, m_1[$$

$$f_2(m) = \left(\frac{C_2}{m}\right)^{(D+\nu)/\nu}, \quad m \in]m_1,\, m_0 + m_1[$$

where C_1 and C_2 are arbitrary constants. The cell balance at m_0 is

$$f_2(m_0 + m_1)\,\nu\,(m_0 + m_1) = f_1(m_0)\,\nu\, m_0$$

and at m_1 is

$$f_2(m_0 + m_1)\,\nu\,(m_0 + m_1) + f_1(m_1)\,\nu\, m_1 = f_2(m_1)\,\nu\, m_1$$

When the solutions are substituted into the two cell balances, the following equations are obtained:

$$m_0\left(\frac{C_1}{m_0}\right)^{(D+\nu)/\nu} = (m_0 + m_1)\left(\frac{C_2}{m_0 + m_1}\right)^{(D+\nu)/\nu}$$

$$(m_0 + m_1)\left(\frac{C_2}{m_0 + m_1}\right)^{(D+\nu)/\nu} + m_1\left(\frac{C_1}{m_1}\right)^{(D+\nu)/\nu} = m_1\left(\frac{C_2}{m_1}\right)^{(D+\nu)/\nu}$$

from which

$$C_1^{(D+\nu)/\nu}\left(\frac{1}{m_0}\right)^{D/\nu} = C_2^{(D+\nu)/\nu}\left(\frac{1}{m_0 + m_1}\right)^{D/\nu}$$

and

$$(m_0 + m_1)^{D/\nu} = (m_1)^{D/\nu} + (m_0)^{D/\nu}$$

which is the desired linkage equation.

We have not assumed any bounds on or relationship between m_0 and m_1, so clearly this result can be valid only if $D/\nu = 1$; i.e., if $D = \nu$, a much simpler linkage equation. Notice that the two parameters m_0 and m_1 do not appear. The specific growth rate of the population depends only on the specific growth rate of single cells. This result is now used to simplify the equation between C_1 and C_2 and eliminate one of these arbitrary constants from the solution for $f(m)$. After a bit of algebra, one obtains

$$f_1(m) = \left(\frac{C_1}{m}\right)^2$$

$$f_2(m) = \frac{m_0 + m_1}{m_0}\left(\frac{C_1}{m}\right)^2$$

As the final step, we will use the normalization criterion to eliminate C_1,

$$1 = \int_{m_0}^{m_1} f_1(m)\ dm + \int_{m_1}^{m_0+m_1} f_2(m)\ dm \quad \Rightarrow \quad C_1^2 = m_0$$

giving

$$f(m) = \begin{cases} \dfrac{m_0}{m^2}, & m \in]m_0,\ m_1[\\ \dfrac{m_0 + m_1}{m^2}, & m \in]m_1,\ m_0 + m_1[\end{cases}$$

Example 3.4: Binary fission with cell death The effect of cell death on growth dynamics will be modeled two different ways. First, we will assume that cell death occurs continuously through the cell cycle with a constant value of the death intensity Θ_c. Next, we will assume that death occurs at discrete control points only. Specifically, we will assume that death occurs only at the division control point with the probability Θ_d. In other words, a cell at this point can either die, probability Θ_d, or divide, probability $1 - \Theta_d$. In both cases we will assume binary division at the state M with birth at the state $M/2$ and first-order single-cell kinetics with the specific growth rate ν.

Continuous case The PBE takes the form

$$\frac{df}{dm} = -\frac{D + \Theta_c + \nu}{\nu\, m} f \quad \Rightarrow \quad f(m) = \left(\frac{C}{m}\right)^{(D+\Theta_c+\nu)/\nu}$$

and the cell balance becomes

$$2 \cdot \nu \cdot M f(M) = \frac{M}{2}\ \nu\ f\left(\frac{M}{2}\right)$$

Substituting the solution for $f(m)$ into the cell balance and simplifying gives the linkage equation between D, ν, and Θ_c:

$$D = \nu - \Theta_c$$

Using this result, the expression for the distribution of states can be simplified to

$$f(m) = \left(\frac{C}{m}\right)^2 = \frac{M}{m^2}$$

where the normalization criterion has been used to obtain the final result. Notice that this result is identical to what one would get without death. This model of cell death thus shows no effect between the rate of death and the shape of the distribution of states. The effect of cell death is solely seen in the linkage equation, which shows that the specific growth rate of the population is depressed relative to the specific growth rate of single cells by an amount equal to the rate of death.

Discrete case The PBE takes the form

$$\frac{df}{dm} = -\frac{D+\nu}{\nu m} f \quad \Rightarrow \quad f(m) = \left(\frac{C}{m}\right)^{(D+\nu)/\nu}$$

and the cell balance becomes

$$2(1-\Theta_d)\nu \cdot M\left(\frac{C}{M}\right)^{(D+\nu)/\nu} = \nu \frac{M}{2}\left(\frac{2C}{M}\right)^{(D+\nu)/\nu}$$

Simplifying gives the linkage equation

$$D = \nu\left(1+\frac{\ln(1-\Theta_d)}{\ln(2)}\right)$$

from which one obtains

$$f(m) = \left(1+\frac{\ln(1-\Theta_d)}{\ln(2)}\right)\frac{M^{1+(\ln(1-\Theta_d))/\ln(2)}}{2^{1+(\ln(1-\Theta_d))/\ln(2)}-1}\left(\frac{1}{m}\right)^{2+(\ln(1-\Theta_d))/\ln(2)}$$

We see from this result that, when cell death is not uniformly distributed over the cell cycle, cell death distorts the shape of the distribution of states relative to the shape found when death does not occur.

In summary, to solve a PBM for the values of the process variables, such as substrate, product concentrations, biomass, and cell concentrations, as functions of the operating parameters, the dilution rate, and substrate feed concentration, one needs the following:

1. The PBE and associated cell balances
2. Substrate/product balances
3. Kinetic equations for the single-cell growth rate parameters in terms of the substrate and product concentrations
4. Expression for the position of the cell cycle control points in terms of substrate and product concentrations

Points 3 and 4 are both equations that model how the single-cell kinetics depend on substrate concentration(s). It is important to realize that the population growth rate depends not only on the single-cell growth rate but also on the locations of the control points. The locations of the control points in state space is therefore an important measure of the single-cell kinetics. For instance, in the age distribution problem, the single-cell growth rate is unity and the population growth rate can change only by changes in the control point(s) for cell division. At higher population growth rates, cell division occurs at younger ages than at low population growth rates. Thus, the linkage equation relating population growth rate and single-cell kinetics becomes an equation between the dilution rate and the division age(s).

One can write all these equations down and solve them simultaneously, but this can be rather confusing, and it is better to work systematically. First solve the PBE for each interval between control points. With N intervals, this gives N arbitrary constants. Then apply the cell balances of which there should be N. The resulting equations can be solved for $N - 1$ of the arbitrary constants in terms of the remaining constant. In addition, one will obtain the linkage equation relating the population growth rate, usually the dilution rate, which equals the specific population growth rate, and the parameters that characterize growth kinetics on the cellular level, i.e., the parameters in the function $r(z)$ and the parameters that specify the location of the control points. The remaining arbitrary constant can be found by using the normalization criterion on $f(z)$.

One can now use the substrate balance to get an equation between the total cell number concentration N and the substrate concentration C_S. However, both are unknown and an additional equation is needed. This equation is the linkage equation. We can formally write this equation as

$$D = F(\nu_1, \nu_2, \ \ldots\ , \nu_M)$$

where ν_m are the single-cell kinetic parameters. But all these parameters are functions of the substrate concentration, so we can write

$$D = F(\nu_1(C_S), \nu_2(C_S), \ \ldots\ , \nu_M(C_S)) \ \Rightarrow \ C_S = F^{-1}(D)$$

The substrate concentration can be found from this equation, and the cell number concentration can then be found from the substrate balance.

3.2 Distributed Breakage Functions

Control point models are obviously quite simplistic, but they do provide reasonable predictions about how the distribution of states changes in shape and position with different growth conditions. The great advantage of control point models is, of course, that the steady-state solutions can almost always be found analytically without any great difficulty.

More realistic models that are based on distributed breakage functions must usually be solved numerically. However, in some special cases, steady-state solutions can be found analytically even if control points are not assumed. It may not seem worthwhile pursuing such models because they lack the simplicity of control point models, yet do not approach the realism of more general models; they thus appear to provide the least advantage of any modeling approach. However, it is the experience of this author that the solutions of these models are very useful as cases against which numerical solutions of general models can be tested. Numerical codes must always be debugged and tested carefully and at a minimum must be able to reproduce the analytical steady-state solutions described in this section when the model parameters and functions are specified such that these analytical solutions are obtainable.

The obvious example of a model with distributed breakage functions that can be solved is the steady-state age distribution case. The solution for the steady-state age distribution, Eqs. (2.12) and (2.13), is

$$f(a) = \frac{e^{-\int_0^a a(D + \Gamma(\bar{a}) + \Theta(\bar{a}))d\bar{a}}}{\int_0^\infty e^{-\int_0^a (D + \Gamma(\bar{a}) + \Theta(\bar{a}))d\bar{a}} da}$$

This case is easy to solve because there is no integral term in the age PBE.

A simplifying assumption that can be used for state parameters other than age is that either birth or division occurs at control points, but not both. We will first consider points for division; in fact, we will assume a single division point for all cells and leave generalization to several discrete points to the reader.

When all cells divide at the same point, say z_d, the division intensity, which equals the rate of division at z_d, is equal to the growth flux into z_d. One can formally write

$$\Gamma(z) = r(z)\,\delta(z - z_d) \tag{3.1}$$

and, since cells never achieve states greater than z_d, the division intensity is not defined for these values. Substituting this expression into the PBE and simplifying, using the elementary properties of the δ-function, gives

$$\frac{df}{dz} = 2r(z_d)f(z_d)\frac{p(z, z_d)}{r(z)} - \frac{D + r'(z)}{r(z)}f(z) \tag{3.2}$$

Solving this equation is a tedious and often difficult task for anything but the simplest kinetic expressions and is illustrated in the example below.

Example 3.5 Let single-cell growth be first order, $r(z) = \nu z$, and let the distribution of birth states be

$$p(z, z_d) = 30\frac{z^2(z_d - z)^2}{z_d^5}$$

We will also assume no death, so it is already known from Eq. (2.11) that $D = \nu$; however, we will rederive this result here. Substituting these expressions into Eq. (3.2) and solving for $f(z)$ subject to the boundary condition that $f(z_d)$ is known gives

$$f(z) = 60\frac{f(z_d)z^4\nu}{(5\nu + D)z_d^4} + 60\frac{f(z_d)z^2\nu}{(3\nu + D)z_d^2} - 120\frac{z^3\nu f(z_d)}{(4\nu + D)z_d^3}$$
$$+ f(z_d)\left(\frac{z_d}{z}\right)^{D/\nu + 1}\frac{D^3 + 12\nu D^2 + 47\nu^2 D - 60\nu^3}{D^3 + 12\nu D^2 + 47\nu^2 D + 60\nu^3}$$

Notice that the last term contains the factor $z^{D/\nu + 1}$, which has a singularity at $z = 0$ (the factor goes to $\pm\infty$ as z goes to 0). This is not acceptable in the solution, so the factor must be multiplied by zero in order for this term to drop out of the solution. Thus we require that

$$D^3 + 12\nu D^2 + 47\nu^2 D - 60\nu^3 = 0$$

which has only one real solution, $D = \nu$, the expected linkage equation for this case. The solution for $f(z)$ simplifies down to

$$f(z) = f(z_d)\left[10\left(\frac{z}{z_d}\right)^4 + 15\left(\frac{z}{z_d}\right)^2 - 24\left(\frac{z}{z_d}\right)^3\right]$$

Applying the normalization criteria

$$\int_0^{z_d} f(z)dz = 1 \Rightarrow f(z_d)z_d = 1$$

Notice that we could also have obtained this result by using Eq. (2.10). At steady state, with $\mu(t) = D$ and with $\Gamma(z)$ given by Eq. (3.1), Eq. (2.10) simplifies to

$$D = r(z_d)f(z_d) = \nu z_d f(z_d) \Rightarrow f(z_d)z_d = 1$$

Consequently

$$f(z) = \frac{1}{z_d}\left[10\left(\frac{z}{z_d}\right)^4 - 24\left(\frac{z}{z_d}\right)^3 + 15\left(\frac{z}{z_d}\right)^2\right]$$

For purpose of comparison, a more narrow distribution of birth states

$$p(z, z_d) = 630\frac{z^4(z_d - z)^4}{z_d^9}$$

gives the solution

$$f(z) = \frac{1}{z_d}\left[126\left(\frac{z}{z_d}\right)^8 - 560\left(\frac{z}{z_d}\right)^7 + 945\left(\frac{z}{z_d}\right)^6 - 720\left(\frac{z}{z_d}\right)^5 + 210\left(\frac{z}{z_d}\right)^4\right]$$

while the control point model with all births occurring at $z_d/2$ gives

$$f(z) = \frac{1}{z_d}\left(\frac{z_d}{z}\right)^2, \qquad \frac{z_d}{2} < z < z_d$$

The three solutions are plotted together in Fig 3.3 in dimensionless form, $z_d f(z)$ versus z/z_d.

The other case that is amenable to analytical solution is the case of distributed cell division but with birth points that are not distributed. In other words, cells split exactly in two halves (or some other ratio) in cell division and the distribution of birth states degenerates to one or more delta functions. When all cells split exactly in half and the state

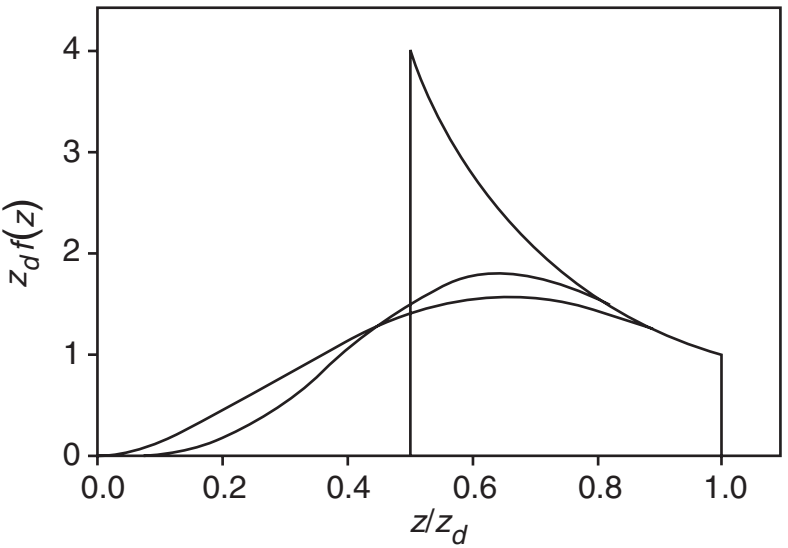

Figure 3.3 Plots of the normalized distributions of states for the three models of binary fission. (It is hopefully obvious to the reader which graph corresponds to which model.)

variable is a quantity that is conserved in division, the function $p(z, \tilde{z})$ takes the form

$$p(z, \tilde{z}) = \delta\,(z - \tilde{z}/2)$$

and the PBE becomes

$$\frac{d\,r(z)\,f(z)}{d\,z} = 4\,\Gamma\,(2z)\,f(2z) - (D + \Gamma\,(z))\,f(z) \tag{3.3}$$

The factor of 4 multiplying the first term on the right-hand side looks suspicious because it seems to imply that four cells, not two, are formed in a division, and this equation has in fact been reported in the literature both with the correct factor of 4 [1, 92] and with an incorrect, but intuitively appealing, factor of 2 [27, 28]. The factor of 4 is not only correct but also physically reasonable once the issue is contemplated. Had we derived the result above by doing a cell balance on cells between z and $z + dz$, then the balance would include a birth flux into the control volume and that birth flux equals twice the division flux of the relevant dividing cells. However, this division flux does not equal $2\Gamma(2z)W(2z)\,dz$, as one might think initially. Since the cell balance is done on cells between z and $z + dz$, the cells that divide into this control volume occupy the state space between $2z$ and $2z + 2dz$, i.e., a state space twice as large as that of the newborn cells. Consequently, the flux of newborn

cells is $2\Gamma(2z)W(2z)d(2z)$ or $4\Gamma(2z)W(2z)dz$. The additional factor of 2 is seen to account for the contraction of state space that follows from division. When the same number of cells is forced to occupy a smaller state space, the distribution function of these cells must increase such that the zeroth moment of the distribution or the cell number does not change.

The PBE we have obtained for binary fission, Eq. (3.3), is a differential, functional equation and is not any easier to solve than the differential, integral equation we started with. However, if one makes the biologically quite reasonable assumption that the cell state parameter z does not exceed some maximum value z_m, then a closed-form solution can in principle be found. Since divisions are binary and the variable z is conserved in a division, cell births do not take place in the interval from $z_m/2$ to z_m. Equation (3.3) therefore does not contain the birth term and can be solved:

$$\frac{dr\,f_1}{dz} = -[D + \Gamma(z)]f_1(z) \Rightarrow \tag{3.4}$$

$$f_1(z) = C_1 \exp\left(-\int_z^{zm} \frac{D + \Gamma(\tilde{z}) + r'(\tilde{z})}{r(\tilde{z})} d\tilde{z}\right), \qquad z_m/2 < z < z_m$$

where prime indicates differentiation with respect to z. The nomenclature here has been changed slightly from that used previously. Instead of counting the interval number, indicated by the subscript, from the lowest value of the state parameter, interval counting now starts at the highest value of the state parameter and counts backward. This numbering is more in tune with the solution method. The solution over the remaining cell states can now be found. For cell states between $z_m/4$ and $z_m/2$, Eq. (3.3) takes the form

$$\frac{dr\,f_2}{dz} = 4f_1(2z)\,\Gamma(2z) - (D + \Gamma(z))f_2(z)$$

$$z \in \left[z_m/4,\ z_m/2\right],\ f_2(z_m/2) = f_1(z_m/2)$$

which has a well-known closed-form solution. In general, the population balance over the interval $[z_m/2^n,\ z_m/2^{n-1}]$ takes the form

$$\frac{dr\,f_n}{dz} = 4f_{n-1}(2z)\,\Gamma(2z) - (D + \Gamma(z))f_n(z)$$

$$z \in \left[z_m/2^n,\ z_m/2^{n-1}\right],\ f_n(z_m/2^{n-1}) = f_{n-1}(z_m/2^{n-1})$$

A cursory examination of the solution method above will make it clear that it does not even require that cell births are discrete in the sense that $p(z, \tilde{z})$ can be represented by δ functions. The method can be used as long as the parameter z is conserved in a division and a minimum and maximum cell state exists. To be more specific, let the minimum and maximum cell states be $z_{\min} \neq 0$ and $z_{\max}$. Certainly, no cell can be born at a state greater than $z_{\max} - z_{\min}$ so in this interval Eq. (3.4) is valid. Now, split the state space into disjoint intervals of the type $[z_{\max} - nz_{\min}, z_{\max} - (n-1)\, z_{\min}]$. The PBEs over these intervals take the form

$$\frac{dr\, f_n}{dz} = -(D + \Gamma(z)) f_n(z) + \sum_{j=1}^{n-1} \int_{z_{\max} - jz_{\min}}^{z_{\max} - (j-1)z_{\min}} p(z, \tilde{z})\, \Gamma(\tilde{z})\, f_j(\tilde{z}) d\tilde{z}$$

Each of these ODEs can be solved in closed form, although it is not easy to see what biologically reasonable choices of $p(z, \tilde{z})$ and $\Gamma(z)$ give results that are easily manageable.

Example 3.6 Let the maximum value of the cell state parameter be $z_m = 2$ and let the division intensity be

$$\Gamma(z) = \begin{cases} 0, & z < 1 \\ \alpha \dfrac{z-1}{2-z}, & z > 1 \end{cases}$$

where α is an adjustable parameter. In this model, the distribution of states splits naturally into two parts: one part between $z = 1$ and $z = 2$ that contains the dividing cells but no births, and another part between $z = 0.5$ and $z = 1$ that contains all the cell births but no divisions. For the dividing cells one obtains

$$f_1(z) = f_1(1)(z(2-z))^{\alpha/2\nu} z^{-D/\nu - 1}, \qquad 1 < z < 2$$

and the differential equation for the second part, between $z = 0.5$ and $z = 1$, becomes

$$\nu \frac{dz\, f_2}{dz} = 4 f_1(1)(2z(2-2z))^{\alpha/2\nu} (2z)^{-D/\nu - 1} \frac{2z-1}{2-2z} - D f_2(z)$$

which must satisfy the continuity boundary condition, $f_2(1) = f_1(1)$. The solution is

$$f_2(z) = \left(1 - \frac{2(4z - 4z^2)^{\alpha/2\nu}}{2^{\frac{D}{\nu}}}\right) \frac{f_1(1)}{z^{D/\nu + 1}}, \qquad 0.5 < z < 1$$

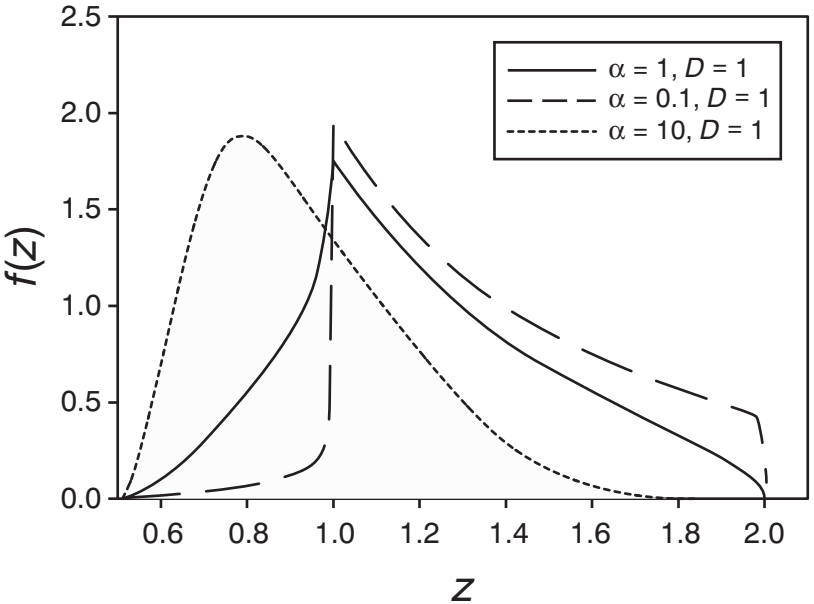

Figure 3.4 Normalized distributions of states.

An additional boundary condition on this solution is that it must equal zero at the minimal value of the state parameters, i.e., $f_2(0.5) = 0$. One finds that

$$f_2(0.5) = 2^{(D+\nu)/\nu} f_1(1) - 4 f_1(1) = 0 \Rightarrow$$
$$D = \nu$$

the expected linkage equation. From this, the solution for $f(z)$ can be written

$$f(z) = f_1(1)\begin{cases} \left(1 - (4z - 4z^2)^{\alpha/2D}\right) z^{-2}, & 0.5 < z < 1 \\ (z(2-z))^{\alpha/2D} z^{-2}, & 1 < z < 2 \end{cases}$$

The constant $f_1(1)$ must be determined from the normalization criteria. This determination turns out to be quite nasty, involving hypergeometric functions, and is probably best done numerically. Figure 3.4 shows the result for several values of α. As expected, as α becomes smaller divisions are less likely to occur at low values of z, occurring predominantly close to $z = 2$, and the distribution approaches the shape of the distribution for the equivalent control point model.

3.3 Problems

3.1 Consider an organism for which division occurs at the cell mass M, birth at the cell mass $M/2$ and for which the single-cell mass growth rate follows first-order kinetics, i.e., $r(m) = \nu m$.

A. Find the normalized, steady-state cell mass distribution $f(m)$ in a chemostat with dilution rate D.

B. Derive the linkage equation between D and ν and use it to eliminate ν from the expression for $f(m)$.

C. Compare the result with the distribution for zeroth-order single-cell kinetics by plotting the two distributions. *Note:* The solution for zeroth-order kinetics is

$$f(m) = \frac{\ln(256)}{M}\exp\left(-\frac{m}{M}\ln(4)\right)$$

D. Assuming a constant yield Y of mass per individual cell per amount of substrate, write a steady-state substrate balance and simplify this to obtain an equation between D, ν, and N, the cell number concentration.

E. Assume that the substrate dependence of ν follows a Monod-type expression. Find the substrate and cell number concentrations as functions of the dilution rate.

F. Assuming that the dependence of M on C_S follows $M = M_0 + \alpha C_S$, solve for M in terms of the two operating parameters D and C_{Sf} and the kinetic parameters of the problem.

3.2 Consider an organism that, when it attains the age a_d, either dies with a probability Θ or divides.

A. Write the cell balance over dividing cells and solve for the steady-state, normalized age distribution in a chemostat with dilution rate D.

B. Show that the doubling time, defined as the duration of the cell cycle a_d does not equal the doubling time defined on the basis of the specific growth rate of the population.

3.3 For plant cell cultures in a chemostat, it has been found that the dilution rate experienced by the cells is different from that of the medium [82]. This is caused by the large size of plant cells and cell clumps, which occasions them to sediment out of the outlet stream and back into the vessel. Of course, this effect can be expected to be more pronounced for large cells than for small cells, and the phenomenon can therefore be expected to affect the cell mass distribution. We can model this by assuming that the cell mass distribution in the outlet stream can be calculated from the distribution in the vessel by the following formula

$$f_{\text{outlet}}(m) = f_{\text{vessel}}(m)\frac{\alpha}{m + \alpha}$$

where α is a constant parameter. Derive a population balance equation for this situation and find the normalized steady-state cell mass distribution in the vessel assuming that: cells divide when they reach the cell mass M, cells are born at the mass $M/2$, and cell mass growth rate follows zeroth-order kinetics. Using α as a parameter, produce a plot with a family of normalized cell mass distributions.

3.4 Cell divisions do not occur at a point in state space but are distributed over a range of ages. We can derive a model that approximates this behavior by assuming that division occurs at a sequence of points in state space. For instance, assume that the cells can divide at the points a_n, given as

$$a_n = a_d + n\Delta a,\ n = 0,\ 1,\ 2,\ \ldots$$

and that at each point a fixed fraction ζ divides while the rest grows older. Find the normalized age distribution for a steady-state chemostat with dilution rate D for this model.

3.5 Budding yeasts such as *Saccharomyces cerevisiae* are organisms for which a single cell state parameter is insufficient to fully describe the cell cycle and the cell cycle controls. The cycle can be described, a bit simplified, as follows: When cells reach a critical cell mass m^*, they initiate budding and all subsequent growth goes into the bud. After attaining the critical mass m^* cell age increases by a fixed amout P before cell division occurs. It follows from this that after cell division, one cell will have the mass m^* and will immediately initiate a new budding cycle. We will refer to the cells with cell masses larger than m^* as mother cells while cells with smaller masses will be called daughters. A convenient way to model the cell cycle is then to use age as a cell state parameter for mothers and cell mass as a parameter for daughters. Thus, daughters become mothers at the cell mass m^*; mothers divide at the age P and at division form a new mother with age 0 and cell mass m^* and a new daughter with a mass equal to the mass at division minus m^*. When a daughter cell attains the mass m^*, it is reclassified as a mother cell with age 0. The cell cycle is sketched in Fig 3.5. In the following, assume that cell mass growth rate follows first-order kinetics, $r(m) = \nu m$, and consider steady-state in a chemostat.

A. Find the mass of newborn daughter cells m_0, and from this determine an upper limit on ν by using the fact that a newborn daughter cell cannot have a mass larger than m^*.

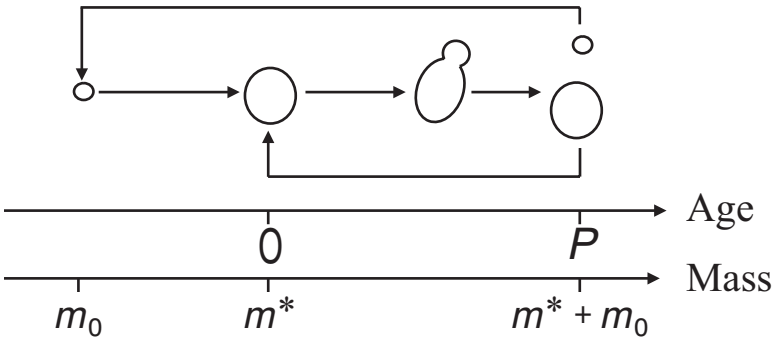

Figure 3.5 Schematic of the budding yeast cell cycle.

B. Write the appropriate population balance equations and cell balances using age as a cell state parameter for mothers and cell mass as a cell state parameter for daughters.

C. Solve for the age distribution of mothers, $f_M(a)$, and the mass distribution of daughters, $f_D(m)$. Find the linkage equation between ν and D and use this result to eliminate ν from the expressions for the distributions.

D. Scale the distributions such that the complete distribution of cell states is unity, i.e.,

$$\int_{m_0}^{m^*} f_D(m)\,dm + \int_0^P f_M(a)\,da = 1$$

and find the fraction of mother cells versus dilution rate.

3.6 Consider an organism for which division occurs at the cell mass M, birth occurs at the cell mass $M/2$, and the single-cell mass growth rate follows $r(m) = k + \nu m$.

A. Find the normalized, steady-state cell mass distribution $f(m)$ in a chemostat with dilution rate D.

B. Find the linkage equation between D and ν and k.

Because there is more than one parameter in the rate expression for the single-cell kinetics, it is not possible to use the last result to simplify the expression for the distribution of states and write it solely as a function of the dilution rate. There are simply not enough equations to solve for ν and k in terms of D. This problem can be resolved if one knows the kinetic equations that give the parameters in terms of the concentration of the growth limiting substrate. From these equations, one can then eliminate the substrate concentration and obtain one of the parameters in terms of the others. This result can then be used with the expression obtained in B to find all the parameters in terms of the dilution rate.

C. Demonstrate this process for the model above by assuming that $k = \bar{k}C_S/(K + C_S)$ and $\nu = \bar{\nu}C_S/(K + C_S)$, where C_S is the concentration of the growth limiting substrate. Express k as a function of ν and substitute the result into the expression obtained in B, solve for ν as a function of D, and eliminate k and ν from the expression for $f(m)$.

3.7 In a cell population, all cells divide when they reach the division age a_d. Over the region of interest, this division age is a function of the concentration of the growth limiting substrate C_S as follows:

$$a_d = \frac{C}{C_S}$$

where C is a known constant. At all cell ages, cell death occurs with a rate given by the constant death intensity Θ. The single-cell yield is independent of cell age, and the substrate balance therefore takes the form

$$\frac{dC_S}{dt} = D(C_{Sf} - C_S) - \int_0^{a_d} \kappa W(a)da$$

where C_{Sf} is the substrate feed concentration, D the dilution rate, κ a known constant, and $W(a)$ the age distribution.

A. Derive an equation for the steady-state value of a_d as a function of dilution rate and substrate feed concentration.

B. Find the steady-state value of the cell number concentration as a function of dilution rate and substrate feed concentration.

3.8 Consider an organism for which all cells divide at the age a_d. This division age is a function of the concentration C_S of the growth limiting substrate, given as $a_d(C_S) = a_0 + K/C_S$. The single-cell yield Y is constant, independent of cell age.

A. Write the chemostat steady-state age population balance equation, the substrate balance, and the renewal equation for this organism.

B. Find the steady-state substrate concentration as a function of dilution rate.

C. Solve for the steady-state cell number concentration.

Chapter

4

Transient Solutions

The one-dimensional, transient PBE for a control point model is

$$\frac{\partial W}{\partial t} + \frac{\partial rW}{\partial z} = -(D + \Theta(z))W$$

or written slightly differently as

$$\frac{\partial W}{\partial t} + r(z)\frac{\partial W}{\partial z} = -(D + \Theta(z) + r'(z))W \tag{4.1}$$

This is a special case of a first-order, homogeneous, linear partial differential equation, a type of equation of the general form

$$\sum_{n=1}^{N} A_n(x)\frac{\partial W}{\partial x_n} = B(x)W(x)$$

where x is a vector of free variables. Structured, transient control point PBEs turn out also to have this form, so the solution methods for solving linear first-order partial differential equations play a key role in the transient solution of PBMs. The complete PBM is obtained when the PBE, valid between the control points, is coupled to cell balances and substrate equations.

The solution method for linear first-order partial differential equations is known as the *method of characteristics* or *Cauchy's method*. The two names refer, not to different methods, but to two different ways of presenting or thinking about what is essentially the same underlying solution method. The method of characteristics presentation is

based on a geometric interpretation of the differential equation and its solution, an interpretation that is very helpful for visualizing the solution method for two-dimensional problems but difficult to use in higher dimensions, i.e., for structured models. Cauchy's method, on the other hand, is more abstract and easily generalized to any number of dimensions. Both methods will be described in this chapter, and, to become fully comfortable with the methods, the reader is urged to study both to the point where it becomes apparent that they both represent the same, identical solution method. The material that follows can range from trivial to quite challenging, depending on the reader's mathematical background. Readers are urged to work through the material by sketching their own diagrams and plots to illustrate the geometric interpretations that underpin the solution methods.

Instead of proceeding straight to the solution method for this type of equation, an example, simple enough to be solved with the use a little physical insight, will be considered first. This will hopefully build an intuitive understanding of the structure of the solutions of these equations.

Example 4.1: The plug-flow reactor, a trivial introductory example A plug-flow reactor is one of several idealized chemical reactors used to develop simple reactor models. It is best visualized as a tube of constant cross section. The mixture of reactants enter at one end of the tube and flow through it with a velocity that is constant and the same for all fluid elements. In other words, the velocity profile of the fluid moving through the tube is flat, like a plug. Thus the name *plug-flow reactor* (PFR). The chemical reactions start when a fluid element enters the reactor and stop when they leave. Each fluid element is assumed isolated from the others; there is no diffusion of matter from one element to another. A differentially small fluid element can therefore be regarded as a tiny batch reactor as it passes through the reactor tube, and the reaction time of this batch reactor equals the time that the fluid element has been inside the plug-flow reactor. One can therefore model a plug-flow reactor by writing a reactant balance on this tiny batch reactor, using a differential control volume that moves with the fluid element, a so-called Lagrangian description. However, one can equally well write a model of the plug flow reactor using a stationary control volume, more specifically a differential slice of the tube, and, in this balance, account for the flux of matter in and out of the control volume due to fluid flow. This is called an *eulerian description*. The two types of models must be equally valid, and one would usually choose to work with the model that is the easiest to solve. But that means that one can solve the more difficult model, say the eulerian model, simply by switching to the simpler model, the lagrangian model. The method of characteristics or Cauchy's method is essentially just that. The methods take a partial differential equation, which represents an eulerian

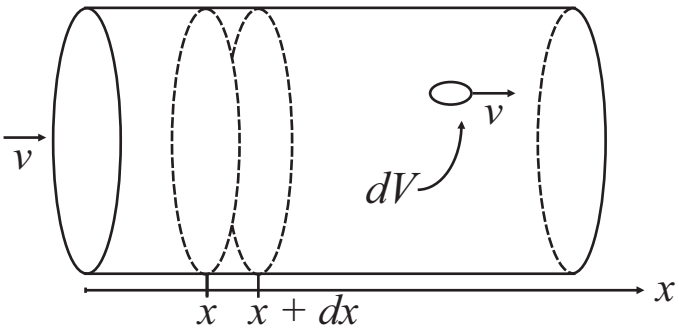

Figure 4.1 Control volumes used in writing balance equations on a PFR. Reactants are moving through the tube with the constant velocity υ. The volume used in an eulerian description is the stationary slice between x and $x + dx$. The volume used in a lagrangian description is the fluid element dV that moves through the reactor with the velocity υ.

description, and changes it to a lagrangian description represented by a set of coupled ordinary differential equations, which can be solved more readily. This will be illustrated by using the plug-flow reactor as an example. A schematic of a plug-flow reactor with the two control volumes that will be used is shown in Fig. 4.1.

Consider a first-order, constant-volume reaction in a plug-flow reactor and let the reactant concentration at position x at time t be $c(t, x)$. To derive a model for this system, we will first use an eulerian description, i.e., a control volume that is fixed in space. Place an x axis along the reactor axis with an origin at the reactor inlet. If the velocity of the fluid through the reactor is υ, a transient balance on the reacting component in a fixed control volume gives

$$\begin{aligned}\text{Flux in by flow} \;-\; &\text{flux out by flow} \;=\\ &\text{rate of accumulation} \;+\; \text{rate of consumption}\end{aligned}$$

or

$$A\upsilon c(x) - A\upsilon c(x + dx) = \frac{dc}{dt} A dx + k c A dx$$

where A is the cross-sectional area of the tube and k is the reaction rate constant. This is rearranged to

$$\frac{\partial c}{\partial t} + \upsilon \frac{\partial c}{\partial x} = -kc \tag{4.2}$$

which is a linear, homogeneous, first-order partial differential equation, the same mathematical form as the PBE between control points. The balance must be supplied with initial and boundary conditions that specify the initial concentration profile in the reactor, $c_0(x)$, $x > 0$, and the inlet concentration $c_{\text{inlet}}(t)$, $t > 0$.

Before solving this partial differential equation, we will write a reactant balance using a lagrangian description. The control volume is a small lump of matter that moves with the fluid flow, and since there is no transfer of matter across the control volume boundary, the reactant balance is simply

$$\text{Rate of accumulation} = -\text{rate of consumption}$$

which results in an ordinary differential equation

$$\frac{dc}{dt} = -kc$$

with the solution

$$c(t) = c_{\text{inlet}}(t_{\text{in}})e^{-k(t-t_{\text{in}})}$$

where t_{in} is the time at which the matter inside the control volume entered the reactor. We would of course like to eliminate this variable and write the solution in terms of t and x only. This is done by using the equation that relates position and time for the fluid element under consideration. Setting the x coordinate equal to zero at the reactor inlet gives

$$x = v(t - t_{\text{in}}) \Rightarrow c(t, x) = c_{\text{inlet}}(t - x/v)^{e-kx/v}$$

The solution, written this way, assumes that the fluid element entered the reactor after the initial time 0 such that the argument of c_{inlet} is positive. If this is not the case, then the initial condition for the fluid element is the initial concentration at the point in the reactor where the fluid element was located at time 0, i.e.,

$$c(t) = c_0(x_{\text{initial}})e^{-kt}$$

but

$$x_{\text{initial}} = x - \upsilon t$$

so

$$c(t, x) = c_0(x - \upsilon t)e^{-kt}$$

and the complete solution to our problem can be written as follows

$$c(t, x) = \begin{cases} c_{\text{inlet}}(t - x/\upsilon)e^{-k(x/\upsilon)}, & x < \upsilon t \\ c_0(x - \upsilon t)e^{-kt}, & x > \upsilon t \end{cases} \tag{4.3}$$

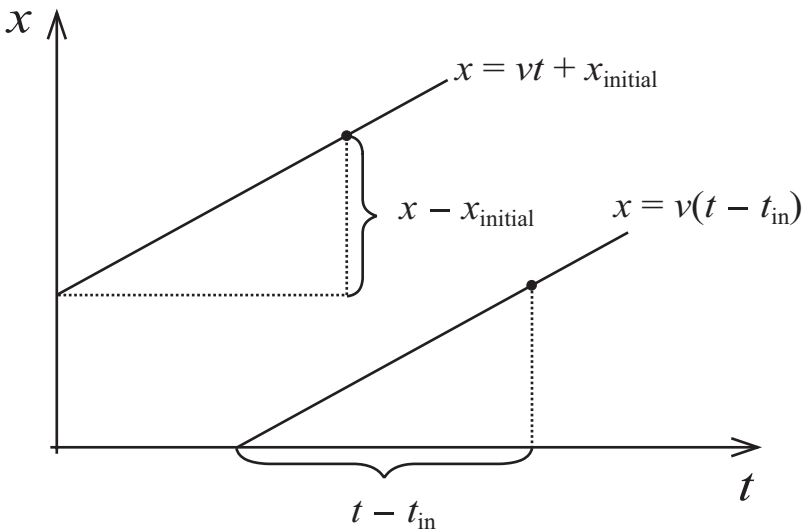

Figure 4.2 Phase space plot of fluid elements in a plug flow reactor.

which, one can easily confirm, is a solution to the eulerian model, Eq. (4.2).

It is instructive to look at a phase space plot of the fluid elements or control volume on which the Lagrangian balance was done. This is shown in Fig. 4.2.

The trajectories of the control volumes are straight lines with a slope of υ, and the initial conditions are given along the positive t and x axis. For there to be a solution along a trajectory, the trajectory must have a point on which an initial condition is known. For trajectories corresponding to fluid elements that were present in the reactor at time zero, this initial point is the point of intersection with the x axis. For the other trajectories, it is the points of intersection with the t axis.

So, we essentially solved the partial differential equation by first converting it to an ordinary differential equation, valid along the trajectories. This equation was then solved and the arbitrary constant evaluated after first determining which initial condition was appropriate by determining if the trajectory intersected with the positive x axis or the positive t axis.

For this example, the ordinary differential equation that was solved along the trajectories, as well as the trajectories themselves, was obtained simply from physical insight into the problem. In the general case a very similar solution procedure is used: An ordinary differential equation is found that must be solved along a family of curves in the state space. For some first-order partial differential equations, one may be able to reformulate the problem in this way by using physical insight; after all, the reformulation is only a change from an eulerian description to a lagrangian description. However, since we cannot rely on being able to do this all the time, we will now look at a systematic way of converting a two-dimensional, first-order partial differential equation problem to a problem involving only coupled ordinary

differential equations. In a sense, we will try to abstract the process that converts an eulerian model to a lagrangian model.

4.1 Method of Characteristics

We will, for illustrative purposes, continue using the partial differential equation for the plug-flow reactor, Eq. (4.2), from the first example. The solution method we will describe is called the *method of characteristics*. Central to the method, as it is presented here, is the concept of a directional derivative, the rate of change, not in the coordinate directions in phase space, but in some other direction, at an angle with the coordinate directions. In order to introduce this concept, we will digress briefly from the main topic.

Consider a curve $C(s)$ in our phase plane (t, x) parameterized by s. For instance, the upper trajectory in Fig. 4.2 can be parameterized the obvious way as $(t(s), x(s)) = (s, \upsilon s + x_{\text{initial}})$. A parameterization of a curve in terms of arc length, starting from an arbitrary point on the curve, is called a *natural parametric representation*. We will indicate it as M : $(t(s), x(s))$ and assume in the following that s is always the arc length.[1]

Given a function $c(t, x)$, one can certainly define a new function $c(s)$ by $c(s) = c(t(s), x(s))$ and ask, What is the rate of change of $c(s)$ with respect to s? From the chain rule,

$$\frac{dc}{ds} = \frac{\partial c}{\partial t}\frac{dt}{ds} + \frac{\partial c}{\partial x}\frac{dx}{ds} = \begin{pmatrix} \dfrac{\partial c}{\partial t} \\ \dfrac{\partial c}{\partial x} \end{pmatrix} \begin{pmatrix} \dfrac{dt}{ds} \\ \dfrac{dx}{ds} \end{pmatrix} \tag{4.4}$$

where the first vector in the scalar product on the right-hand side is the gradient of $c(t, x)$ and the second vector is the positive unit tangent vector to the curve k. (A proof of the second fact can be found in any elementary textbook on differential geometry.) It is important in this context that the curve is parameterized by the arc length, because only when this is the case is the vector $(dt/ds, dx/ds)$ a unit vector, and it therefore holds that $(dt/ds)^2 + (dx/ds)^2 = 1$. Comparing this equation to the well-known formula $\cos(\alpha)^2 + \sin(\alpha)^2 = 1$, where α can be any

[1]Notice that the parametric representation of a curve is not unique. For instance, the upper trajectory in Fig. 4.2 can just as easily be parameterized by the expressions $(t(s), x(s)) = (s^3, \upsilon s^3 + x_{\text{initial}})$ or $(t(s), x(s)) = (\sinh(s), \upsilon \sinh(s) + x_{\text{initial}})$. Not even the natural parametric representation is unique because the starting point for measuring the arc length is arbitrary. In general, it is relatively easy to find some parametric representation of a curve, while finding a natural parametric representation is surprisingly difficult. So, although a natural parametric representation of a curve is rarely found, the existence of such a representation has important theoretical significance. Readers wanting to learn more about this can consult any introductory text on differential geometry.

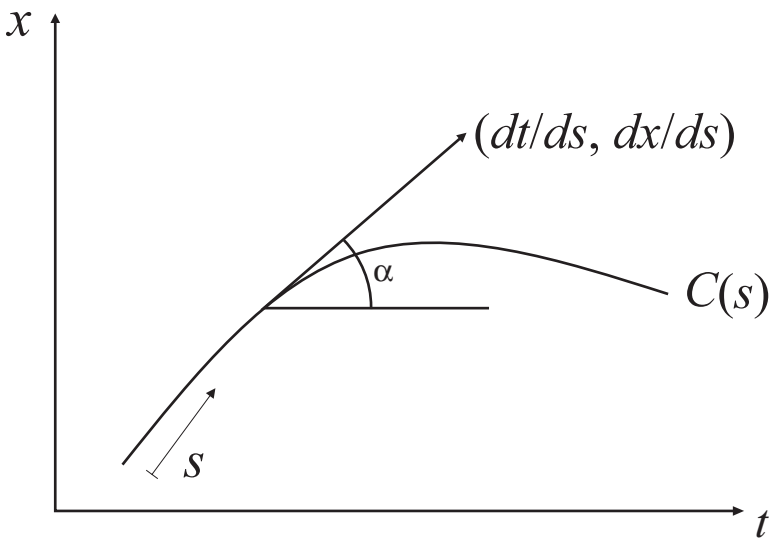

Figure 4.3 Positive unit tangent vector $(dt/ds,\ dx/ds)$ to the curve $C(s)$ and the directional angle α.

number, it can be seen that one can always define a so-called directional angle α such that

$$\left(\frac{dt}{ds},\ \frac{dx}{ds}\right) = (\cos(\alpha),\ \sin(\alpha))$$

The directional angle α is the angle between the t axis and the positive tangent vector (Fig. 4.3). In terms of this angle, the expression for dc/ds becomes

$$\frac{dc}{ds} = \frac{\partial c}{\partial t}\cos(\alpha) + \frac{\partial c}{\partial x}\sin(\alpha)$$

We now want to try and rewrite the eulerian model of the plug-flow reactor, Eq. (4.2), so that it resembles the form above, in which the coefficient functions can be interpreted as directional cosines. This is done by dividing through by $\sqrt{1+\upsilon^2}$:

$$\frac{\partial c}{\partial t} + \upsilon\frac{\partial c}{\partial x} = -kc \Rightarrow$$

$$\frac{1}{\sqrt{1+\upsilon^2}}\frac{\partial c}{\partial t} + \frac{\upsilon}{\sqrt{1+\upsilon^2}}\frac{\partial c}{\partial x} = -\frac{k}{\sqrt{1+\upsilon^2}}c$$

and one can now clearly interpret the two coefficient functions as directional cosines because they satisfy

$$\left(\frac{1}{\sqrt{1+\upsilon^2}}\right)^2+\left(\frac{\upsilon}{\sqrt{1+\upsilon^2}}\right)^2=1$$

so one can define

$$\cos(\alpha)=\frac{1}{\sqrt{1+\upsilon^2}}=\frac{dt}{ds}$$

$$\sin(\alpha)=\frac{\upsilon}{\sqrt{1+\upsilon^2}}=\frac{dx}{ds}$$

giving the model equation

$$\frac{dt}{ds}\frac{\partial c}{\partial t}+\frac{dx}{ds}\frac{\partial c}{\partial x}=-\frac{k}{\sqrt{1+\upsilon^2}}c$$

and by comparison with Eq. (4.4), we see that the eulerian model of the plug-flow reactor can be written as

$$\frac{dc}{ds}=-\frac{k}{\sqrt{1+\upsilon^2}}c$$

We now have an ordinary differential equation for the dependent variable and one could solve this for $c(s)$, find the natural parametric representation for the curves $(t(s), x(s))$, and eliminate s to obtain $c(t, x)$. The dedicated reader, who will spend 15 minutes doing this as an important part of the learning process, will discover that all the algebraic work leads to a cancellation of the square root term in the solution for $c(s)$. In fact, the ordinary differential equation above is not the most convenient equation to work with. A simpler problem is almost always obtained if the arc length s is eliminated from the problem before the equation is solved. To do this, we will multiply both sides of the equation by ds/dt and assume without proof that $ds/dt = 1/(dt/ds)$. Thus

$$\frac{dc}{ds}\frac{ds}{dt}=\frac{-kc}{\sqrt{1+\upsilon^2}}\sqrt{1+\upsilon^2}=-kc$$

The quantity on the left-hand side is known as a directional derivative, indicated

$$\left.\frac{dc}{dt}\right|_{\alpha}$$

It represents the rate of change of c with respect to t when c changes along a line that makes an angle α with the t axis. Thus, one can write

$$\left.\frac{dc}{dt}\right|_{\alpha} = -kc \tag{4.5}$$

and we see that all the result of all the manipulations is to reduce the sum of the partial derivatives on the left-hand side of Eq. (4.2) to a single derivative, creating an ordinary differential equation. The right-hand side of Eq. (4.2) is unchanged.

Solution of Eq. (4.5) requires an initial condition, and the solution that is found is not simply $c(t, x)$ but c along the curve $C(s)$ (which is not yet known). Assuming an initial condition is known at the point (t_0, x_0), the solution can be written

$$c(t,\ x) = c(t_0,\ x_0)\, e^{-k(t-t_0)} \qquad \text{along curve} \tag{4.6}$$

The curves in the (t, x) plane along which c is obtained can formally be written as $t(x)$ or $x(t)$, and they must be found by solving another ordinary differential equation, this time in t and x. This equation is obtained as follows:

$$\frac{dt}{dx} = \frac{dt}{ds}\frac{ds}{dx} = \frac{1}{\sqrt{1+\upsilon^2}}\frac{\sqrt{1+\upsilon^2}}{\upsilon} = \frac{1}{\upsilon} \Rightarrow$$

$$t = \frac{x}{\upsilon} + p \tag{4.7}$$

The solutions form a one-parameter family of curves, parameterized by p, which arises naturally as the arbitrary constant in the integration of the differential equation. The solution curves are called the *characteristic curves* or *characteristic base curves* or *characteristic ground curves*.

To find the final solution at a point (t, x), one must piece together information about the characteristic curves, the solution for the dependent variable along the characteristic curves and the initial and boundary conditions for the partial differential equation. Usually, this is straightforward but tedious. One proceeds as follows:

1. For the given point, (t, x), determine the characteristic curve on which this point lies. If the family of characteristic curves is given by $F(t, x, p) = 0$, then this means determining the value of the parameter p as a function of t and x.
2. Then find the point on this characteristic curve where an initial condition is known. Call this point $(t_0(p(t, x)), x_0(p(t, x)))$. If such a unique point cannot be identified, then the problem is not well posed

and does not have a solution. At this point, the initial conditions provide a value for c, say

$$c(t_0(p(t,\ x),\ x_0(p(t,\ x))) = c_0(p(t,\ x))$$

3. Finally, the solution to our problem is obtained as

$$c(t,\ x) = c_0(t_0(p(t,\ x)),\ x_0(p(t,\ x)))e^{-k(t-t_0(p(t,\ x)))}$$

It is obvious that initial and boundary conditions are treated in an identical fashion and usually they are both called initial conditions because they both function as initial conditions to the ordinary differential equation along the characteristics [Eq. (4.5)]. The curve in the phase space along which the initial/boundary conditions are given is called the *initial manifold*.

To illustrate this process for the plug-flow reactor problem, note that the initial manifold is the positive t and x axis and the family of characteristic curves is parameterized by p and given by Eq. (4.7). To find the solution at a given point (t, x), first solve for p:

$$p = t - \frac{x}{\upsilon}$$

If p is greater than zero, then the characteristic curve through (t, x) intersects the initial manifold at the positive t axis; otherwise, it intersects the manifold at the positive x axis. Consider first the case when $p > 0$, or equivalently, $\upsilon t > x$. In this case, p has a simple physical interpretation; it is the time at which the lump of matter, which at time t is at position x, entered the reactor. The characteristic curve intersects the t axis at the point $(t_0, x_0) = (p, 0) = (t - x/\upsilon, 0)$, and the initial condition at this point is $c_{\text{inlet}}(t_0) = c_{\text{inlet}}(t - x/\upsilon)$. Substituting all this into Eq. (4.6) gives

$$\begin{aligned} c(t,\ x) &= c_{\text{inlet}}(t - x/\upsilon)e^{-k(t-(t-x/\upsilon))} \\ &= c_{\text{inlet}}(t - x/\upsilon)\mathrm{e}^{-k(x/\upsilon)} \qquad \upsilon t > x \end{aligned}$$

which is identical to the previously found result, Eq. (4.3).

Consider now the case when $x > \upsilon t$ and $p < 0$. The characteristic curves through (t, x) now intersect the initial manifold on the positive x-axis at the point $(t_0,\ x_0) = (0, -\upsilon p)$ where the initial condition is $c(x_0) = c(-\upsilon p) = c_0(x - \upsilon t)$, giving

$$c(t, x) = c_0(x - \upsilon t)e^{-kt}, \quad x > \upsilon t$$

which is identical to the previously found result, Eq. (4.3).

We will now retire our plug-flow reactor example and try to use this method for an equation of the more general form

$$p(t, x)\frac{\partial c}{\partial t} + q(t, x)\frac{\partial c}{\partial x} = r(t, x)c + f(t, x)$$

Assuming that $p \neq 0$ and $q \neq 0$, the equation can be rewritten as

$$\frac{p}{\sqrt{p^2+q^2}}\frac{\partial c}{\partial t} + \frac{q}{\sqrt{p^2+q^2}}\frac{\partial c}{\partial x} = \frac{r}{\sqrt{p^2+q^2}}c + \frac{f}{\sqrt{p^2+q^2}} \Rightarrow$$

$$\frac{dc}{ds} = \frac{r}{\sqrt{p^2+q^2}}c + \frac{f}{\sqrt{p^2+q^2}}$$

where s is the arc length along the characteristic curves. Remember that we will interpret the new coefficient functions as

$$\frac{p}{\sqrt{p^2+q^2}} = \frac{dt}{ds}$$

$$\frac{q}{\sqrt{p^2+q^2}} = \frac{dx}{ds}$$

One can now multiply this equation by either ds/dt or ds/dx. It does not matter which one, since the two free variables appear in a completely symmetric fashion in the equation. For argument's sake, multiply by ds/dt to get

$$\frac{dc}{ds}\frac{ds}{dt} = \frac{rc}{\sqrt{p^2+q^2}}\frac{\sqrt{p^2+q^2}}{p} + \frac{f}{\sqrt{p^2+q^2}}\frac{\sqrt{p^2+q^2}}{p} \Rightarrow$$

$$\left.\frac{dc}{dt}\right|_{\alpha} = \frac{rc}{p} + \frac{f}{p}$$

This differential equation is now solved and the solution is valid along the characteristic curves which are found from

$$\frac{dx}{dt} = \frac{dx}{ds}\Big/\frac{dt}{ds} = \frac{q}{p}$$

Finally, the arbitrary constants must be determined from the initial values given along the initial manifold.

4.2 Cauchy's Method

The description of the method of characteristics given above relies on the concept of a directional derivative, which is basically a geometric concept and therefore hard to generalize to higher dimensions—at least without losing the insight that the geometric interpretion provides. Therefore, Cauchy's method, a more abstract formulation of the method of characteristics that is easily generalized to higher dimensions, will now be presented. Consider again a linear first-order partial differential equation of the form

$$p(t,\ x)\frac{\partial c}{\partial t} + q(t,\ x)\frac{\partial c}{\partial x} = r(t,\ x)c + f(t,\ x) \tag{4.8}$$

with an initial condition given on the initial manifold M. As the first step, parameterize the initial manifold by the parameter τ. This parameter does not have to be the arc length, but can be the parameter that gives the simplest and most convenient parameterization of M. We can then formally write the initial condition as

$$c(t(\tau),\ x(\tau)) = c_0(\tau)$$

along M. Define now the so-called characteristic equations

$$\frac{dT}{ds} = p(T(s,\ \tau),\ X(s,\ \tau)), \qquad T(0,\ \tau) = t(\tau)$$

$$\frac{dX}{ds} = q(T(s,\ \tau),\ X(s,\ \tau)), \qquad X(0,\ \tau) = x(\tau)$$

$$\frac{dC}{ds} = r(T(s,\ \tau),\ X(s,\ \tau))C(s,\ \tau) + f(T(s,\ \tau),\ X(s,\ \tau)), \qquad C(0,\ \tau) = c_0(\tau)$$

These equations are first-order, coupled ordinary differential equations that in the general case are obtained as follows. For the first two equations, take the coefficient functions of Eq. (4.8), i.e., $p(t, x)$ and $q(t, x)$, and convert these to functions of one argument s by setting each free variable, t and x, equal to a function of s. Then set the derivative (with respect to s) of the nth free variable equal to the nth coefficient function. The equations obtained this way are called the *base equations* and their solutions are the characteristic curves. The dependent variables of the base equations, $T(s)$ and $X(s)$, are indicated by capital letters to distinguish them from the free variables, t and x, of Eq. (4.8). However, it will be shown shortly that T can be identified with t and X with x. Because of this identification, many texts do not distinguish clearly between the two types of variables and use the same set of symbols for both. We will adopt this convention in the examples

that follow, after we have proved that it is valid to do so. To be able to identify the free variables of Eq. (4.8) with the solutions to the base equations, the initial conditions to the base equations must be the points on the initial manifold. These points are given in terms of the parameter τ, and the solutions to the base equations are therefore functions of both s and τ. To emphasize this fact, some people write the derivative in the base equations using partial differentials.

The last characteristic equation is slightly different from the base equations. It is obtained from the right-hand side of Eq. (4.8) in a similar fashion to that of the base equations. The initial condition is the initial values of the dependent variable along the initial manifold, and is thus a function of τ.

We will first show that, if $c(t, x)$ is a solution to Eq. (4.8) and T is identified with t and X with x, then

$$c(T(s),\ X(s)) = C(s, \tau)$$

To see this, simply calculate the derivative of $c(T(s,\ \tau), X(s,\ \tau))$ with respect to s and confirm that it satisfies the defining equation for $C(s, \tau)$. Using the chain rule, one first obtains

$$\frac{d}{ds}(c(T(s, \tau),\ X(s, \tau)) = \frac{\partial c}{\partial t}\frac{dT}{ds} + \frac{\partial c}{\partial x}\frac{dX}{ds}$$

where, in order to be able to use the chain rule, I have identified $T(s, \tau)$ with the free variable t and $X(s, \tau)$ with the free variable x. (When using the chain rule, one can equally well make the opposite identification, but that would not be a good idea. Why?) Then, using the base equations, one gets

$$\frac{d}{ds}(c(T(s, \tau),\ X(s, \tau)) = \frac{\partial c}{\partial t}p(T(s, \tau),\ X(s, \tau)) + \frac{\partial c}{\partial x}q(T(s, \tau),\ X(s, \tau))$$

but since $c(t, x)$ is a solution to Eq. (4.8), the right-hand side can be rewritten to obtain

$$\frac{d}{ds}(c(T(s, \tau),\ X(s, \tau)) = r(T(s, \tau),\ X(s, \tau))c(s, \tau) + f(T(s, \tau),\ X(s, \tau))$$

which is the defining equation for $C(s, \tau)$. Thus, $c(T(s, \tau), X(s, \tau)) = C(s, \tau)$ is a solution to Eq. (4.8) and, furthermore, a solution to the initial value problem, provided that $c(T(0), X(0)) = c_0(\tau)$.

To eliminate s and τ from the problem, one first solves the characteristic equations to obtain T and X as functions of s and τ. But, as mentioned above when the chain rule was used, $T(s, \tau)$ is identified with

t and $X(s, \tau)$ is identified with x, so the following equations must hold between these variables:

$$\frac{dt}{ds} = \frac{dT}{ds},\ T(s = 0,\ \tau) = t(s = 0,\ \tau)$$
$$\frac{dx}{ds} = \frac{dX}{ds},\ X(s = 0,\ \tau) = x(s = 0,\ \tau)$$

Solving for x and t gives

$$x = X(s,\ \tau),\ t = T(s,\ \tau)$$

Invert these equations to obtain

$$\tau = \tau(t,\ x),\ s = s(t,\ x)$$

and substitute this result into the expression for $C(s, \tau)$:

$$c(t,\ x) = C(s(t,\ x),\ \tau(t,\ x))$$

When formulated this way, the method of characteristics is easily generalized to problems of any dimensionality. We will state this general procedure before considering specific examples.

Given:

$$\sum_{n=1}^{N} A_n(x) \frac{\partial c}{\partial x_n} = B(x)c(x) + D(x)$$

where x is a vector of free variables, x_n. The initial values of $c(x)$ are given along the initial manifold M. The solution is obtained as follows.

1. Parameterize the initial manifold and initial condition by the parameters $\tau_1,\ \tau_2, \ldots,\ \tau_{N-1}$. We can formally write this as

$$M : (x_1(\tau),\ x_2(\tau),\ \ldots,\ x_n(\tau))\, c_0 = c_0(\tau)$$

   where τ is the vector $(\tau_1, \tau_2, \ldots, \tau_{N-1})$.

2. Write and solve the N coupled ordinary differential equations that constitute the characteristic equations, using the initial conditions given along the initial manifold

$$\frac{d}{ds} X_n(s,\ \tau) = A_n(X(s,\ \tau)),\ X_n(0,\ \tau) = x_n(0,\ \tau)$$

3. Set

$$x = X(s, \tau)$$

and invert these equations to obtain

$$s = s(x), \ \tau = \tau(x)$$

This inversion can be a very difficult problem if the dimensionality of the problem is high.

4. Solve the ODE

$$\frac{d}{ds}C(s, \tau) = B(X(s, \tau))\, C(s, \tau) + D(X(s, \tau))$$

subject to the initial condition

$$C(0, \tau) = c_0(\tau)$$

5. The solution of the PDE then is

$$c(x) = C(s(x), \tau(x))$$

Example 4.2: The plug-flow reactor strikes back For first-order reaction kinetics, the concentration of a reactant in a plug-flow reactor is given by Eq. (4.2):

$$\frac{\partial c}{\partial t} + \upsilon \frac{\partial c}{\partial x} = -k c$$

Let the initial conditions be given as

$$c(0, x) = c_0(x), \ x > 0$$
$$c(t, 0) = c_{in}(t), \ t > 0$$

and solve for the concentration using Cauchy's method.

First parameterize the initial condition along the positive x axis as

$$t = 0 : x = \tau, \ c = c_0(\tau)$$

From the base equations we get

$$\left.\begin{aligned} &\frac{dt}{ds} = 1, \ t(0) = 0 \Rightarrow t = s \\ &\frac{dx}{ds} = \upsilon, \ x(0) = \tau \Rightarrow x = \upsilon s + \tau \end{aligned}\right\} \Rightarrow \begin{aligned} s &= t \\ \tau &= x - \upsilon t \end{aligned}$$

The last characteristic equation is

$$\frac{dc}{ds} = -kc,\ c(0) = c_0(\tau) \Rightarrow$$

$$c(s, \tau) = c_0(\tau)e^{-ks} \Rightarrow$$

$$c(x, t) = c_0(x - \upsilon t)e^{-kt}$$

Repeat now this procedure for the initial condition given at the reactor inlet. Parameterize the initial condition as

$$x = 0 : t = \tau,\ c = c_{in}(\tau)$$

Write and solve the base equations

$$\left.\begin{aligned} \frac{dt}{ds} &= 1,\ t(0) = \tau \Rightarrow t = s + \tau \\ \frac{dx}{ds} &= \upsilon,\ x(0) = 0 \Rightarrow x = \upsilon s \end{aligned}\right\} \Rightarrow \begin{aligned} s &= x/\upsilon \\ \tau &= t - x/\upsilon \end{aligned}$$

and the last characteristic equation

$$\frac{dc}{ds} = -kc,\ c(0) = c_{\text{in}}(\tau) \Rightarrow$$

$$c(s, \tau) = c_{\text{in}}(\tau)e^{-ks},\ \Rightarrow$$

$$c(x, t) = c_{\text{in}}(t - \frac{x}{\upsilon})e^{-kx/\upsilon}$$

At this point in the presentation, it is probably a good idea to point out two common sources of confusion and error that occur in working with Cauchy's method. The first source of confusion is caused by the commonly used nomenclature for functions of several variables. This nomenclature is very poor and usually leaves a lot to be inferred from the context, but it is used because an unambiguous, rigorous nomenclature is too cumbersome to work with. Consider, for instance, the function $f = x \sin(y)$. We can make the list of free variables explicit two different ways: either as $f(x, y) = x \sin(y)$ or as $f(y, x) = x \sin(y)$. Often, we may use $f(x, y)$ and $f(y, x)$ inter-changeably because the free variables are physical quantities, amplitude and phase in this example, and we "know" that the amplitude x always appears as factor and the phase y as the argument of the sine function. However, this careless permutation of arguments must be avoided when physical insight is missing and the order in which the free variables are given in the argument list becomes important. For instance, if we define $f(x, y) \equiv x \sin(y)$, then this definition states that the first argument in the list appears as a factor of the sine function and the second argument as the argument of the sine function; i.e., $f(y, x) = y \sin(x)$. The order of

the arguments as they appear in an expression for a function can be made explicit by indexing the arguments by the natural numbers; i.e., $f = x_1 \sin(x_2)$. This indexing also makes it possible to define derivatives in an unambiguous way. For instance, even after we define $f(x, y) \equiv x \sin(y)$, the expression $\partial f / \partial x$ remains ambiguous because we do not know if the x is a reference to the first argument of f or to a free variable called "x" that can appear as either the first or second argument of f. An unambiguous definition of a derivative would be $\partial f / \partial x_n$ which is the derivative of f with respect to the nth variable. We will not use this unwieldy indexing of the free variables in the text that follows. However, we will assign significance to the position of a variable in the argument list and argument can therefore not be casually permuted.

Another possible source of error is the confusion of dummy variables in integrals with a true variable. Consider, for instance, the problem

$$\frac{\partial W}{\partial x} + \frac{\partial W}{\partial y} = F(x, y)W$$

subject to an initial condition along the x axis:

$$\mathcal{M} : x = \tau, \; y = 0, \; W = W_0(\tau)$$

Proceeding as usual, the base equations give

$$\left.\begin{aligned} \frac{dx}{ds} &= 1 \;, \quad x(0) = \tau \;\Rightarrow\; x = s + \tau \\ \frac{dy}{ds} &= 1 \;, \quad y(0) = 0 \;\Rightarrow\; y = s \end{aligned}\right\} \Rightarrow \begin{aligned} s &= y \\ \tau &= x - y \end{aligned}$$

and

$$\frac{dW}{ds} = F(\tau + s, s)W(s) \;, \; W(0) = W_0(\tau) \;\Rightarrow$$

$$W(s, \tau) = W_0(\tau) e^{\int_0^s F(\tau + s, s) ds}$$

One must now recognize that the s that appears inside the integral is a dummy variable, while the s that appears in the upper limit of the integral is a true variable. Thus, when the expressions for s and τ that were obtained from the base equations are substituted into this result, the substitution should not be made in the dummy variable, i.e.,

$$W(x, y) = W_0(x - y) e^{\int_0^y F(x - y + s, s) ds}$$

To minimize the risk of mistakes, it often pays to use unique symbols for dummy variables as soon as they appear; e.g., write the solution to the last characteristic equation as

$$W(s,\ \tau) = W_0(\tau)e^{\int_0^s F(\tau+\bar{s},\ \bar{s})d\bar{s}}$$

The next example has no relevance to population balances whatsoever. In fact, it is a deliberate attempt to present an example that is as far removed from population balances as possible in order to provide additional practice with Cauchy's method as an abstract tool. Readers who feel that they do not need this additional practice can skip the example without loss of continuity.

Example 4.3: The wave equation Just as some nth-order ordinary differential equations can be solved by reducing the problem to the solution of n first-order equations, some higher-order partial differential equations can be solved by reducing them to the solution of several first-order equations. An elegant example of this technique is d'Alembert's solution of the wave equation. The wave equation is

$$\frac{\partial^2 W}{\partial t^2} - \gamma^2 \frac{\partial^2 W}{\partial x^2} = 0$$

Initial conditions are usually given as initial displacement and initial velocity:

$$W(x,\ 0) = f(x), \qquad \left(\frac{\partial W}{\partial t}\right)_{(x,\ 0)} = g(x)$$

This second-order wave equation can be written as two first-order differential operators, operating in series on W:

$$\left(\frac{\partial}{\partial t} + \gamma\frac{\partial}{\partial x}\right)\left(\frac{\partial}{\partial t} - \gamma\frac{\partial}{\partial x}\right) W = 0$$

Remember that the expressions in the parentheses are operators and the parentheses are not the common algebraic kind. Instead, the expression above means that all the derivatives inside a pair of parentheses operate on the expression to the right of the parentheses. Thus all the derivatives inside the first pair operate on everything inside the second pair and the derivatives inside the second pair operate on W. If we call the argument of the outer operator U, we have

$$\frac{\partial U}{\partial t} + \gamma\frac{\partial U}{\partial t} = 0$$

and

$$U = \frac{\partial W}{\partial t} - \gamma \frac{\partial W}{\partial x} = 0$$

The initial condition on U is

$$U(x, 0) = g(x) - \gamma f'(x)$$

We start by solving for U. First parameterize the initial manifold and initial conditions by

$$x = \tau \ : t = 0, \qquad U = g(x) - \gamma f'(x)$$

The characteristic equations become

$$\frac{dx}{ds} = \gamma, \qquad \frac{dt}{ds} = 1, \qquad \frac{dU}{ds} = 0$$

Solving these subject to the initial conditions gives

$$x = \gamma s + \tau \ , \ t = s \ , \ U(s, \tau) = g(\tau) - \gamma f'(\tau)$$

Now solve for s and τ and substitute into the result for $U(s, \tau)$:

$$s = t \ , \ \tau = x - \gamma t \ \Rightarrow$$
$$U(x, t) = g(x - \gamma t) - \gamma f'(x - \gamma t)$$

From the definition of U, one then obtains a new PDE problem:

$$\frac{\partial W}{\partial t} - \gamma \frac{\partial W}{\partial x} = g(x - \gamma t) - \gamma f'(x - \gamma t) \ , \ W(x, 0) = f(x)$$

The initial conditions are parameterized as

$$x = \tau \ , \ t = 0 \ , \ W = f(\tau)$$

The characteristic equations are

$$\frac{dx}{ds} = -\gamma, \quad \frac{dt}{ds} = 1, \quad \frac{dW}{ds} = g(x(s, \tau) - \gamma t(s, \tau)) - \gamma f'(x(s, \tau) - \gamma t(s, \tau))$$

and their solution is

$$x = -\gamma s + \tau \ , \ t = s$$
$$W = \int_0^s (g(x(s, \tau) - \gamma t(s, \tau)) - \gamma f'(x(s, \tau) - \gamma t(s, \tau)))ds + f(\tau)$$

Both g and f' are functions of only one argument $x - \gamma t$. From the solution for x and t we find that this argument can be written as $x - \gamma t = -2\gamma s + \tau$. We can therefore write the solution for $W(s, \tau)$ as

$$W(s,\ \tau) = \int_0^s (g(-2\gamma s + \tau) - \gamma f'(-2\gamma s + \tau))ds + f(\tau)$$
$$= -\frac{1}{2\gamma}\int_{\tau}^{-2\gamma s + \tau} g(\lambda)d\lambda + \frac{1}{2}f(-2\gamma s + \tau) + \frac{1}{2}f(\tau)$$

where the variable transformation $\lambda = -2\gamma s + \tau$ has been used to obtain the last integral. We can now proceed as usual: Solve for s and τ in terms of x and t and substitute this result into the expression for $W(s, \tau)$ to get d'Alembert's solution:

$$W(x, t) = \frac{1}{2}(f(x + \gamma t) + f(x - \gamma t) + \frac{1}{2\gamma}\int_{x-\gamma t}^{x+\gamma t} g(\lambda)d\lambda$$

In the next example, we will solve a simple instance of the transient age distribution problem. The problem is special in that it does not assume any control points of the cell cycle but uses a general division intensity.

Example 4.4: Solution of the transient age distribution The age distribution PBE is

$$\frac{\partial W}{\partial t} + \frac{\partial W}{\partial a} = -(D + \Gamma(a, t))W(a, t)$$

with the initial condition and the renewal equation.

$$W(a, 0) = W_0(a) \quad W(0,\ t) = 2\int_0^{\infty} \Gamma(a, t)\ W(a, t)\ da$$

Even if we assume that $\Gamma(a, t)$ is given, such that this PDE problem is uncoupled from the substrate balances, it is still difficult to solve because the initial condition along that part of the initial manifold that is the time axis is given by the renewal equation and not by some specified, known function. To overcome this problem, we will solve for each generation in turn and find the entire age distribution as the sum over all generations. Let $w_n(a, t)$ be the age distribution of cells that have undergone n divisions since time 0 and try to find a closed-form expression for these distributions. Clearly

$$W(a, t) = \sum_{n=0}^{\infty} w_n(a, t)$$

where w_n satisfies the PBE and the following side conditions:

$$w_0(a, 0) = W_0(a), \quad w_n(0, t) = 2\int_0^{\infty} w_{n-1}\Gamma(a, t)\ da\ ,\ n > 0$$

For w_0, the initial manifold is the positive age axis while, for the other distributions, it is the positive time axis. The solution for the zeroth distribution is obtained separately from the others. Parameterizing the initial conditions for w_0 along the age axis gives

$$a = \tau, \quad t = 0, \quad w_0(0, \tau) = W_0(\tau)$$

Solve the base equations to get

$$\left.\begin{aligned} \frac{da}{ds} &= 1, \quad a(0) = \tau \Rightarrow a = s + \tau \\ \frac{dt}{ds} &= 1, \quad t(0) = 0 \Rightarrow t = s \end{aligned}\right\} \Rightarrow \begin{aligned} s &= t \\ \tau &= a - t \end{aligned}$$

The third characteristic equation can now be written and solved:

$$\frac{dw_0}{ds} = -(D + \Gamma(s + \tau, s))w_0(s), \; w_0(0) = W_0(\tau) \Rightarrow$$

$$w_0(s, \tau) = W_0(\tau)e^{-Ds}e^{-\int_0^s \Gamma(\tilde{s} + \tau, \tilde{s})d\tilde{s}}$$

So finally

$$w_0(a, t) = W_0(a - t)e^{-Dt}e^{-\int_0^t \Gamma(\tilde{s} + a - t, \tilde{s})d\tilde{s}}$$

The solution for the remaining distributions is now obtained in the same straightforward manner. Initial conditions are parameterized along the positive time axis as follows:

$$t = \tau, \qquad a = 0, \qquad w_n(0, \tau) = 2\int_0^\infty w_{n-1}(a, \tau)\Gamma(a, \tau)\, da$$

Solve the base equations

$$\left.\begin{aligned} \frac{da}{ds} &= 1, \qquad a(0) = 0 \;\Rightarrow\; a = s \\ \frac{dt}{ds} &= 1, \qquad t(0) = \tau \;\Rightarrow\; t = s + \tau \end{aligned}\right\} \Rightarrow \begin{aligned} s &= a \\ \tau &= t - a \end{aligned}$$

and the last characteristic equation becomes

$$\frac{dw_n}{ds} = -(D + \Gamma(s, s + \tau))w_n(s), \quad w_n(0) = 2\int_0^\infty w_{n-1}(a, \tau)\Gamma(a, \tau)da$$

giving

$$w_n(s,\ \tau) = 2\int_0^{\infty} w_{n-1}(\tilde{a},\ \tau)\,\Gamma(\tilde{a},\ \tau)\,d\tilde{a}\ e^{-Ds}e^{-\int_0^{s}\Gamma(\tilde{s},\,\tilde{s}+\tau)\,d\tilde{s}} \Rightarrow$$

$$w_n(a,\ \tau) = 2\int_0^{\infty} w_{n-1}(\tilde{a},\ t-a)\,\Gamma(\tilde{a},\ t-a)\,d\tilde{a}\ e^{-Da}e^{-\int_0^{a}\Gamma(\tilde{s},\,\tilde{s}+t-a)\,d\tilde{s}}$$

The following cumbersome expression is then obtained for the closed-form solution of the age distribution:

$$W(a,\ t) = W_0(a-t)e^{-Dt}e^{-\int_0^{t}\Gamma(\tilde{s}+a-t,\,\tilde{s})\,d\tilde{s}} +$$

$$\sum_{n=1}^{\infty} 2^n \int_0^{\infty}\cdots\int_0^{\infty} W_0(a_1-t)\prod_{m=1}^{n} e^{-Da_m}e^{-\int_0^{a_m}\Gamma(\tilde{s},\,\tilde{s}+t-a_m)\,d\tilde{s}}\,\Gamma(a_m,\ t)\,da_m$$

The generational approach used in the above example to solve for the transient age distribution can be extended to other, more complicated, models [54]. Derivation of the method, as described above, is based on a biological argument but can be given a sound theoretical basis in terms of integral equations. To recast the age population balance as an integral equation, note that a solution for $W(0, t)$ is just as good as a solution for $W(a, t)$, since the latter can always be found from the former by considering the entire t axis as the initial manifold. Thus, we find that

$$W(a,\ t) = W(0,\ t-a)\ e^{-\int_0^{a} D+\,\Gamma(s,\,t-a+s)\,ds}\,da$$

Substitution into the renewal equation gives

$$\begin{aligned} W(0,\ t) &= 2\int_0^{\infty}\Gamma(a,\ t)W(0,\ t-a)e^{-\int_0^{a} D+\,\Gamma(s,\,t-a+s)\,ds}\,da \\ &= \int_{-\infty}^{t} 2\,\Gamma(t-s,\ t)e^{-\int_0^{t-s} D+\,\Gamma(s,\,s+s)\,ds}\,W(0,\ s)\,ds \\ &= \int_{-\infty}^{t} K(t,\ s)W(0,\ s)\,ds \end{aligned}$$

We can now use the initial condition that specifies $W(0, t)$ for negative values of t to rewrite this result as

$$\begin{aligned} W(0,\ t) &= \int_{-\infty}^{0} K(t,\ s)W(0,\ s)\ ds + \int_0^{t} K(t,\ s)\ W(0,\ s)\ ds \\ &= f(t) + \int_0^{t} K(t,\ s)\ W(0,\ s)\ ds \end{aligned}$$

where both $K(t, s)$ and $f(t)$ are known functions. The above equation is a well-known problem in mathematics; it is known as a Volterra integral equation of the second kind. This kind of integral equation is solved by the method of successive approximations, a method similar to the well-known Picard iteration used for solving first-order ordinary differential equations. The zeroth-order approximation to the solution is the inhomogeneous term $f(t)$:

$$w_0(t) = f(t)$$

and the higher-order approximations are then found recursively as

$$w_n(t) = f(t) + \int_0^t K(t, s)\ w_{n-1}(s)ds$$

The solution is obtained as

$$W(0, t) = \lim_{n \to \infty} w_n = f(t) + \sum_{n=1}^{\infty} \int_0^t K_n(t, s) f(s)\, ds$$

where the kernels that appear in the integrals are defined recursively as

$$K_n(t, s) = \int_0^t K(t, s) K_{n-1}(t, s) ds$$

The series that appears in the solution is called a Neumann series.

Obviously, the treatment of integral equations above is quite brief and does not address important questions such as whether or not the successive approximations or the Neumann series converge. Interested readers can consult almost any book on integral equations to find a deeper discussion of these issues.

4.3 Fixed Control Points

Looking back at the transient PBE, Eq. (4.1), it is clear that the characteristic curves are given by the differential equation

$$\frac{dz}{dt} = r(z)$$

and the characteristic curves are therefore the single-cell growth curves. Keeping this in mind is helpful when solving the transient population balance. The process for doing so is sketched in Fig. 4.4. The solution is obtained in a repetitive process in which one first solves the PBE in an initial domain and then uses this solution together with the

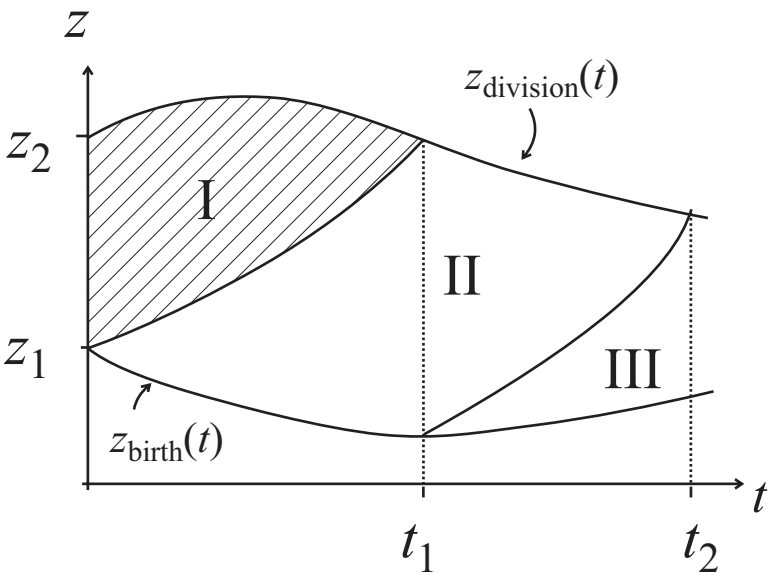

Figure 4.4 Solution process for transient PBMs. The solution in the hatched domain, I, is found from the initial condition. This solution, in conjunction with the cell balance over division and birth, then provides an initial condition for the solution in domain II.

cell balances or boundary conditions to generate the initial conditions for a new domain [43].

In Fig. 4.4, the region of state space in which cells are found is limited from below by the state of cells at birth, the curve $z_{birth}(t)$, and from above by the state of cells at division, the curve $z_{division}(t)$. It is possible to encounter more complex cases than the one depicted here, cases for which the state of cells at birth is not the lower bound on the cell containing region in state space. This will occur whenever the rate of single-cell growth is less than the rate of change of the state at birth. In such a situation, an additional domain below the curve $z_{birth}(t)$ is formed. However, the solution procedure is not significantly altered by this additional domain.

As the initial population of cells grow older, their growth curves cover the domain marked I in Fig. 4.4. Of course, this domain is the same as the domain covered by the characteristic curves that intersect the z axis at time zero and the solution for the transient distribution of states in the domain is readily obtainable from the initial condition. The domain vanishes at time t_1 when the youngest of the cells present at time zero divides.

Transient Solutions

The rate of cell division versus time is then calculated from the solution in domain I, and a cell balance provides the value of the rate of cell birth as a function of time up to the time t_1. This function, along the initial manifold $z_{\text{birth}}(t)$, then serves as the initial condition for the problem in domain II. Continuing in this fashion, the solution in domain II is used to calculate an initial condition for domain III, etc. To keep the nomenclature user-friendly, it is a good idea to indicate the domain as a subscript on the distribution of states, i.e., label the individual domain solutions W_{I}, W_{II}, etc.

The transient problem becomes particularly simple if the control points are fixed. In that case, the control point balances are the same as for the steady-state case and the solution of the transient problem proceeds in a straightforward manner. This is illustrated in the next example.

Example 4.5: Binary fission Consider an organism that divides at the cell mass M and for which all new cells are formed at the cell mass $M/2$. Assume further that single-cell mass growth rate is zeroth order with rate constant k. Given an initial mass distribution $W_0(m)$ for this organism, find the mass distribution after one cell cycle period when it is cultivated in a chemostat with dilution rate D.

The cell cycle period obviously equals $M/(2k)$. The model equations are the PBE between the control points

$$\frac{\partial W}{\partial t} + k\frac{\partial W}{\partial m} = -DW$$

and the cell balance over the control point

$$W(M/2, t) = 2W(M, t)$$

and the initial condition

$$W(m, 0) = W_0(m)$$

The solution domain structure for this problem is very simple and is sketched in Fig. 4.5. Domain I is bounded from above by the division control point $m = M$ and from below by the growth curve $m = M/2 + kt$. Domain II is bounded from above by this growth curve and below by the birth control point $m = M/2$.

In domain I, parameterize the initial manifold by

$$t = 0, \quad m = \tau, \quad W = W_0(\tau)$$

The characteristic equations become

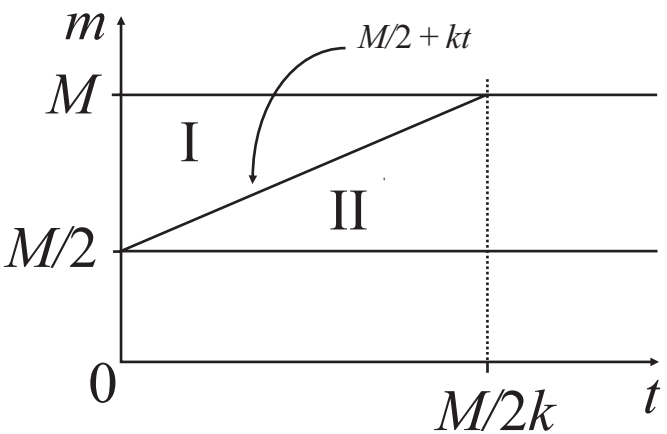

Figure 4.5 The solution domains for a binary fission organism with zeroth-order single-cell mass growth, division at M, and birth at $M/2$.

$$\frac{dm}{ds} = k, \quad \frac{dt}{ds} = 1, \quad \frac{dW}{ds} = -DW(s)$$

which are solved to give

$$m = ks + \tau, \quad t = s, \quad W(s, \tau) = W_0(\tau)e^{-Ds}$$

Inverting the first two of these equations to find τ and s as functions of m and t gives

$$\tau = m - kt, \quad s = t$$

Substituting this result into the expression for $W(s, \tau)$ gives the solution in domain I:

$$W_{\mathrm{I}}(m, t) = W_0(m - kt)e^{-Dt}$$

We can now proceed to domain II. The initial manifold for this domain is the line segment $m = M/2$, $t \in [0, M/(2k)]$, and the initial condition along the manifold is given by a cell balance as

$$W_{\mathrm{II}}(M/2, t) = 2W_{\mathrm{I}}(M, t)$$
$$= 2W_0(M - kt)e^{-Dt}$$

Parameterize this as

$$t = \tau, \quad m = M/2, \quad W_{\mathrm{II}} = 2W_0(M - k\tau)e^{-D\tau}$$

The characteristic equations are

$$\frac{dm}{ds} = k, \quad \frac{dt}{ds} = 1, \quad \frac{dW_{II}}{ds} = -DW_{II}(s)$$

and the solutions become

$$m = ks + M/2, \quad t = s + \tau, \quad W_{II}(s, \tau) = 2W_0(M - k\tau)e^{-D\tau}e^{-Ds}$$

Inverting the two first equations gives

$$s = (m - M/2)/k, \quad \tau = t - (m - M/2)/k$$

and substituting into the result for W_{II} gives

$$W_{II}(m, t) = 2W_0(M/2 + m - kt)e^{-Dt}$$

So the mass distribution after one cell cycle period becomes

$$W(m, M/(2k)) = 2W_0(m)e^{-DM/2k}$$

Notice that the shape of the distribution has not changed, only its magnitude.

4.4 Transient Control Point Balances

As already mentioned, the cell balances or boundary conditions that must be used in transient calculations are, in general, not the same as those that are used in steady-state calculations. Situations in which the birth and/or division state(s) change with time therefore present special problems. The reason for this is that the cell balances are obtained from expressions for the cell fluxes into and out of cell cycle control points and when the position of these points change with time, the fluxes depend on both the growth flux and the rate of change of the control point. The obvious solution to this is to set the flux of cells into the control point equal to the difference of the growth flux and the rate of change of the control point—in other words, to use the growth rate relative to the control point velocity. This is correct in almost all cases. To determine exactly when it is correct, we derive this result more rigorously.

Consider the situation shown in a phase space plot in Fig. 4.6.

A cell that at time zero is in the state z reaches the control point state Z at time t. Clearly, the total number of cells that divide between time 0 and time t is simply the integral of the cell number distribution of states from $z(0)$ to $Z(0)$. The average cell flux into the control point between time zero and t therefore is

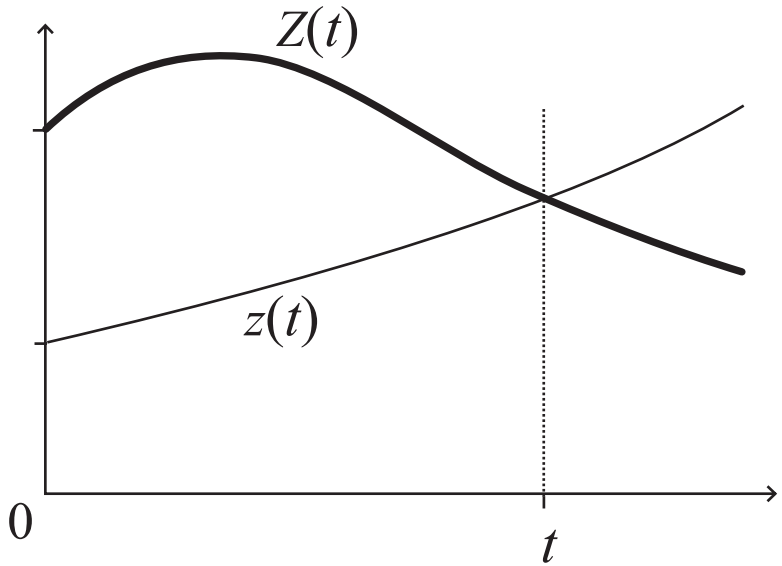

Figure 4.6 Calculation of cell flux into a moving control point. The growth curve of the cell, $z(t)$, is indicated by the thin line; the trajectory of the control point, $Z(t)$, is indicated by the thick line. The total number of cells that divide between time zero and time t is equal to the number of cells present at time zero with states z greater than $z(0)$ — in other words, the integral of the distribution of states from $z(0)$ to $Z(0)$. The average flux into the control point is this integral divided by t.

$$\text{Average cell flux into control point} = \frac{\int_{z(0)}^{Z(0)} W(t, z)\ dz}{t}$$

so the cell flux at time 0 is the limit of this expression.

$$\text{Cell flux into control point at time 0} = \lim_{t \to 0} \frac{\int_{z(0)}^{Z(0)} W(t, z)\ dz}{t}$$

$$= -\lim_{t \to 0} \frac{\int_{z(t)}^{Z(t)} W(t, z) dz - \int_{z(0)}^{Z(0)} W(t, z) dz}{t}$$

$$= -\left(\frac{d}{dt}\right)_{t=0} \int_{z(t)}^{Z(t)} W(t, z) dz$$

$$= \left(r(z(0)) - \left(\frac{dZ}{dt}\right)_{t=0}\right) W(0, z)$$

or

$$\text{Flux into control point} = \left(r(Z) - \left(\frac{dZ}{dt}\right)\right)W(t, Z) \tag{4.9}$$

We see that when the control point moves with time, the factor $r(Z)$ must be replaced with $r(Z) - dZ/dt$, where Z is the state of the control point. This is, in fact, the above-mentioned relative growth flux. If dZ/dt is greater than $r(Z)$, then the flux is formally negative and, of course, a negative flux does not occur. When $dZ/dt > r(Z)$, then the magnitude of the division state increases faster than the single cell growth rate so the cells cannot "keep up" with the increase and cell division simply ceases temporarily.

Similarly, if a birth control point moves as $Z(t)$, then the flux out of the control point is

$$\text{Flux out of control point} = \left(r(Z) - \left(\frac{dZ}{dt}\right)\right)W(t, Z)$$

and this flux is also negative if $dZ/dt > r(Z)$. However, a negative birth flux does make physical sense. It simply means that the single cell growth curves that cross the $Z(t)$ curve do so "from above." Stated differently: The newborn cells grow so slowly that their growth curves lie below $Z(t)$, and growth curves that are above $Z(t)$ will, with time, intersect $Z(t)$, causing a growth flux into the moving control point. A cell balance on such a point must therefore account for two fluxes into the point, the growth flux of cells with states immediately above $Z(t)$ and the birth flux from dividing cells. The sum of these two fluxes equals the growth flux out of the point.

Some of these scenarios will be illustrated in the examples that follow.

The derivation above is valid only when the integral of the distribution of states is differentiable, and this is not the case if the distribution is discontinuous or specifically a δ function. Only the δ function case is worth considering because discontinuities do not show up in the cell balances, as they appear only along the boundaries that separate the solution domains. However, δ functions represent a pulse of perfectly synchronized populations, which we will study in a later section. For these populations we will not need to write flux balances as before, but instead we must find the instant in time when the synchronized population reaches the control point. At this point we can then do a balance on all the cells represented by the δ function. Depending on what happens at the control point, the pulse of cells can either continue at the other side of the control point, it can divide to form pulses of newborn synchronized cells, or it can merge with a pulse

of newborn synchronized cells if these cells are born at the exact time when the first pulse reaches the birth control point.

In solving transient problems, it is extremely helpful to first produce a reasonably accurate sketch of the solution domains. This is usually possible without having to solve the PBE because the domain boundaries are characteristic curves that are often known or easily found if the single cell growth kinetics is known. Thus, in order to sketch the solution domains in state space, one needs the trajectories of the control points and the growth curves that form the domain boundaries. When a domain boundary intersects the curve of division states, the boundary terminates (assuming all cells divide), but is propagated to the cells formed in the division. Thus a new domain boundary emanates from the curve of birth states at the same point in time that the old domain boundary terminates at the curve of division states.

In this context, it is useful to define the concept of a cell line. By a cell line, we mean the growth curves or trajectories in state space of a group of identical cells and their offspring. Because domain boundaries propagate in cell divisions to both daughter cell states, the domain boundaries of a given problem are identical to one or several cell lines. To find all the domain boundaries one must identify all the cell lines that correspond to boundaries. We have already seen that such cell lines arise from the youngest cells in the initial population, but a domain bounding cell line can also appear if the mathematical expression for a cell balance changes at a point in time. This will, for instance, occur if the state at division or the state at birth is modeled by using different functional expressions in different time intervals. When this happens, the initial manifold along which the cell balance is applied must be split into two parts, the first part corresponding to the cell balance before the change and the second part to the cell balance after the change. A domain bounding cell line arises at the point where the change occurs and this cell line must be included when sketching the solution domains of the problem.

In the next couple of examples we will consider situations in which the control points move in state space as part of the transient behavior.

Example 4.6: Shift up in dilution rate for binary fission organism An increase in dilution rate will force the cells to divide more rapidly and the age at division must therefore decrease in some fashion after a dilution rate shift-up. The actual transient behavior of the division age will depend in some complex way on the growth kinetics and the past history of the culture, but we can hope to capture much of the essential features of the population dynamics by modeling the division transient by the simplest possible model: a linear decrease with time from the old to the new value followed by a constant function

equal to the division age at steady state under the new dilution rate. Thus, if a_d is the age at division, we have the model

$$a_d = \begin{cases} a_1 - (a_1 - a_2)\frac{t}{\delta}, & t < \delta \\ a_2, & t > \delta \end{cases}$$

where the shift-up occurred at time 0. It is readily found from solution of the steady-state problem that the two division ages a_1 and a_2 are related to the dilution rates by

$$D_n a_n = \ln 2$$

and, furthermore, the initial condition for this problem is

$$W_0(a) = 2N_0 D_1 e^{-D_1 a}$$

In the calculations that follow, N_0 is carried along as a constant multiplier, so to avoid writing this constant all the time, we will simply set it equal to 1 and remember that in this problem W actually stands for W/N_0.

In sketching a diagram of the solution domains, a problem immediately materializes: The way that the solution domains tile the state space is sensitive to the value of δ. Two possible scenarios are shown in Fig. 4.7.

Because the mathematical expression for the age at division changes at δ, we are forced to consider cells that divide before δ separately from cells that divide after, and the cell line that originates at the point $(\delta, 0)$ will therefore be a solution domain boundary. Specifically, the boundaries are the growth curves that pass through the points $(\delta + na_2, 0)$ where n is any nonnegative integer. Similarly, the cell line originating with the youngest cell in the initial population will be a domain bounding cell line. The boundaries formed by this cell line are the growth curves that pass through the points $(na_2, 0)$. In the top diagram of Fig. 4.7, δ equals a_2 and the two sets of domain boundaries are superimposed. In the bottom part, δ is less than a_2. Notice that half as many domains per unit time are formed when δ equals a_2 than when δ is different from a_2. Since the domain tiling depends on the value of δ, it is difficult to derive a general closed-form solution with δ as an arbitrary parameter. In the rest of the problem, we will therefore first focus on the special case when $\delta = a_2$.

In domain I, the solution to the age distribution balance is

$$\begin{aligned} W_I(t, a) &= W_0(a - t)e^{-D_2 t} \\ &= 2D_1 \, e^{-D_1 a - t(D_2 - D_1)} \end{aligned}$$

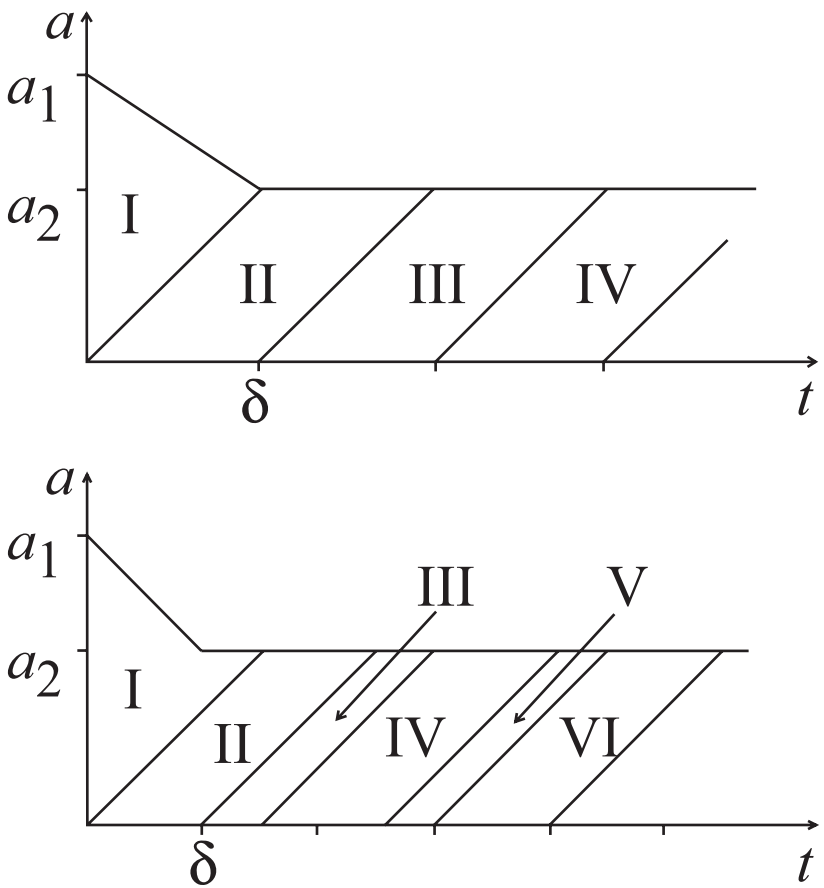

Figure 4.7 The solution domains in state space that are generated when the age at division decreases linearly with time between zero and δ and remains constant thereafter. The top diagram shows the situation when δ equals the new steady-state age at division a_2; the bottom diagram shows the situation when δ is less than a_2. The diagram for the situation when δ is greater than a_2 is left as an exercise.

and the boundary condition along $t \in]0, a_d[$ becomes

$$W_{\mathrm{II}}(t, 0) = 2W_{\mathrm{I}}\left(t, a_d\right)\left(1 - \frac{da_d}{dt}\right)$$

$$= 4D_1 e^{-D_1\left(a_1 - \left(a_1 - a_2\right)/a_2 t\right) - t\left(D_2 - D_1\right)\frac{a_1}{a_2}}$$

$$= 2D_2$$

where all the simplifications have been done using $a_1 D_1 = a_2 D_2 = \ln 2$. The solution in domain II can now be found as

$$W_{II}(t, a) = 2D_2 e^{-D_2 a}, \qquad \delta = a_2$$

but this is simply the steady-state solution at the new dilution rate! Further calculations are unnecessary, the distribution will obviously not change from its steady-state value. This certainly is remarkable, the transient dies out completely in the period of just one cell cycle. However, this is not a clue offering a deep insight into population dynamics; it is only a remarkable coincidence, as we can discover by solving the case when δ equals $a_2/2$. Before proceeding any further, the reader should draw a reasonably accurate diagram of the solution domains for this case and find the points of intersection between the domain boundaries and the age at division. This diagram will make it easier to follow the solution procedure below. We start by finding the solution in the first domain, the domain spanned by the cells present at the initial time. This is obviously the same as the solution in the previous case,

$$W_I(t, a) = 2D_1 e^{-D_1 a - t\left(D_2 - D_1\right)}$$

but now the cell balance takes a different form for $t < a_2/2$ and for $t > a_2/2$. In the first case

$$\begin{aligned} W_{II}(t, 0) &= 2W_I\left(t, a_d\right)\left(1 + 2\frac{a_1 - a_2}{a_2}\right) \\ &= 4D_1\left(2\frac{a_1}{a_2} - 1\right) e^{-D_1\left(a_1 - 2\left(a_1 - a_2\right)t/a_2\right)} e^{-t\left(D_2 - D_1\right)}, \qquad t \in]0, a_2/2[\end{aligned}$$

which can be simplified to

$$W_{II}(t, 0) = 2D_1\left(2\frac{D_2}{D_1} - 1\right) e^{t\left(D_2 - D_1\right)}, \qquad t \in]0, a_2/2[$$

giving the following solution in domain II

$$W_{II}(t, a) = 2D_1\left(2\frac{D_2}{D_1} - 1\right) e^{t\left(D_2 - D_1\right) - a\left(2D_2 - D_1\right)}, \qquad \delta = a_2/2$$

while in the second case

$$W_{III}(t, 0) = 2W_I\left(t, a_2\right) = 4D_1 e^{-D_1 a_2 - t\left(D_2 - D_1\right)}, \qquad t \in]a_2/2, a_2[$$

giving

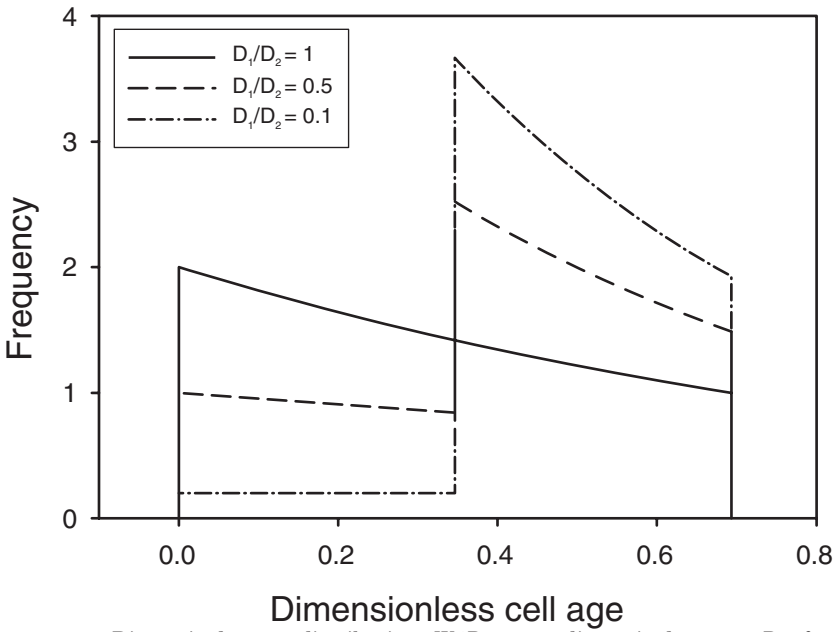

Figure 4.8 Dimensionless age distributions W/D_2 versus dimensionless age aD_2 after a dilution rate shift-up. The parameters used are $\delta = a_2/2$ and $t = a_2$.

$$W_{\mathrm{III}}(t,\ a) = 4D_1 e^{-\left(D_1 / D_2\right)\ln 2 - t\left(D_2 - D_1\right) - \mathrm{a}\, D_1}, \qquad \delta = a_2 / 2$$

We can now find the solution at time $t = a_2$ as

$$W\left(\mathrm{t} = a_2,\ a\right) = \begin{cases} W_{\mathrm{III}}\left(a_2,\ a\right), & a < a_2 / 2 \\ W_{\mathrm{II}}\left(a_2,\ a\right), & a > a_2 / 2 \end{cases}$$

and using that $a_2 D_2 = \ln 2$ this can be simplified to

$$W\left(a_2,\ a\right) = \begin{cases} 2 D_1 e^{-aD_1}, & a < a_2 / 2 \\ 4 D_1 \left(2D_2 / D_1 - 1\right) e^{-\ln 2 \left(D_1 / D_2\right) - a\left(2 D_2 - D_1\right)}, & a > a_2 / 2 \end{cases}$$

This solution becomes very different from the steady-state solution when the difference between the two dilution rates becomes large, as is shown in Fig. 4.8, where the dimensionless age distribution is plotted versus dimensionless age. We see that as the change in dilution rate becomes greater, a larger fraction of the population ends up in domain II. In other words, the population is partially synchronized by the dilution rate shift and the degree

of synchrony increases when the difference between the two dilution rates increases.

One can often get a good idea of the qualitative character of the transient simply by studying the way in which the solution domains tile the state space. For instance, consider again the tiling shown in the bottom diagram of Fig. 4.7, the shift-up in growth rate. At the time $t = a_2$, the solution has two parts, the younger cells in domain III and the older cells in domain II. This solution is plotted in Fig. 4.8, and one can see that the frequency of older cells, domain II cells, has increased relative to that of younger cells, domain III cells. How can one conclude that this will happen just by studying the domain tiling in Fig. 4.7?

Simply note that the domain II cells are descendents of cells that divided while the age of division decreased with time, while the domain III cells are descendents of cells that divided while the division age was constant. Cells that divide while the division age decreases with time experience a contraction of state space similar to the contraction seen when single-cell growth curves converge. The contraction occurs because the newborn cells must fit in a more narrow age interval than the dividing cells. For the example in question, we can be quite specific and state that the cells that divide while the division age is a decreasing function of time, initially at time 0, have ages from $a_2 - \delta$ to a_1. After one division, at time δ, the daughter cells fit in the age interval from 0 to δ. Thus, the width of the state space of these cells lines decrease from $a_1 - a_2 + \delta$ to δ. Just as for converging single-cell growth curves, the contraction of state space causes the frequency to increase, and this is why one can conclude, just from looking at the domain tiling in Fig. 4.7, that the frequency in domain II, the domain containing cells that have experienced a state space contraction, must have increased relative to the frequency of cells in domain III, the domain containing cells that did not experience a contraction.

One can go further and conclude that this increase in frequency becomes more extreme as δ becomes smaller. In the limit as δ goes to zero, all cells between ages a_2 and a_1 divide simultaneously at time zero, causing the state space of these cells to contract to size zero and giving rise to a perfectly synchronized pulse of cells, which must be modeled as a δ function.

The effect of the contraction of state space can also be seen in Eq. (4.9). When the birth state decreases with time, the term dZ/dt is negative and the factor $r - dZ/dt$, which multiplies W, becomes greater than r, causing the flux into the moving control point to increase above the value it would have for a stationary control point. This flux increase is then reflected in an increase in the value of the frequency after the division. Similarly, it can be seen that an increase in the value of the

division state, $dZ/dt > 0$, will cause an expansion of state space in a division resulting in a relative decrease in the frequency after the division.

Contraction and expansion of state space will also occur if the magnitude of a birth control point changes with time. The reader is urged to ponder this situation and reason out what qualitative dynamics is to be expected in different situations.

In the examples done up till now, the tiling of state space by the solution domains was relatively simple because, even though all domains were not the same size, the tiling had a nice repetitive or periodic structure and the domain size did not decrease with time. It is, however, quite easy to construct situations in which this is not the case: where the domain tiling is a periodic and the domain size is continuously decreasing. This situation can, for instance, occur in organisms with unequal division such as budding yeasts. In these organism, the unequal division causes a domain boundary or cell line to split into two different lines in a division, one for each type of newborn cell. So, unless these cell lines later merge, an event that will only occur in unusual circumstances, the number of domain boundaries in the distribution of states will increase approximately exponentially with time, making an analytical solution at large times practically impossible to obtain except in special cases (cases that will be pursued in the next section). In the next example, we will consider such a situation.

Example 4.7: Shift up of budding yeast The cell cycle of Baker's yeast, or *Saccharomyces cerevisiae*, a budding yeast, can be summarized, somewhat simplified, as follows. At division, a large and a small cell are formed. The larger is termed a *mother cell* and the younger a *daughter cell*. All newborn mother cells have the same cell mass m^* and immediately initiate budding, a process that takes a fixed amount of time, P. During this period, all growth goes into formation of the bud, which at cell division becomes the daughter cell with mass m_0. This newborn daughter cell mass must equal the mass of the budded mother cell at division minus m^*, and newborn daughter cells must grow to attain the cell mass m^* before they can initiate their first budding cycle. The cell cycle is sketched in Fig. 4.9.

For this organism, it is convenient to use cell age as the state parameter for the mother cell and cell mass as the state parameter for daughter cells. Assuming that single-cell mass growth follows first-order kinetics, the PBEs for a batch culture become

$$\frac{\partial W_{\mathrm{M}}}{\partial t} + \frac{\partial W_{\mathrm{M}}}{\partial a} = 0$$

$$\frac{\partial W_{\mathrm{D}}}{\partial t} + \nu\, m \frac{\partial W_{\mathrm{D}}}{\partial m} = -\nu\, W_{\mathrm{D}}$$

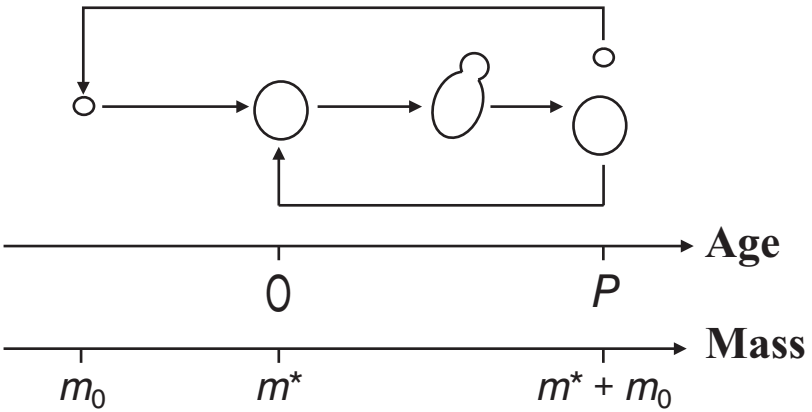

Figure 4.9 Schematic of the budding yeast cell cycle.

where the subscripts M and D refer to mother and daughter cells, respectively, and ν is the specific single-cell mass growth rate. The first transient cell balance gives the rate of formation of mother cells at mass m^* as the sum of a birth flux from dividing mother cells and a growth flux of daughter cells that attains the mass m^*. The second sets the flux out of the control point for daughter cell birth equal to the flux into the division point:

$$W_M(t, 0) = \left(1 - \frac{dP}{dt}\right)W_M(t, P) + W_D\left(t, m^*\right)\left(\nu(t)m^*(t) - \frac{dm^*}{dt}\right)$$
$$\left(\nu(t)m_0(t) - \frac{dm_0}{dt}\right)W_D\left(t, m_0(t)\right) = \left(1 - \frac{dP}{dt}\right)W_M(t, P) \tag{4.10}$$

where the newborn daughter cell mass is

$$m_0(t) = m^*\left(e^{\int_{t-P}^{t} \nu(\tau)d\tau} - 1\right)$$

Let the initial distributions be $W_{M0}(a)$ and $W_{D0}(m)$. To pin the problem down further, we will restrict ourselves to solving for the transient after a step increase in ν. We will render all units of time dimensionless by division with P and all cell masses dimensionless by division with m^*. Effectively, we accomplish this by setting both P and m^* equal to 1. Further, let the dimensionless specific growth rates before and after the growth rate shift, ν_1 and ν_2 respectively, equal

$$\nu_1 = 1/3 \qquad \nu_2 = 1/2$$

It is now easy to show that the dimensionless birth mass changes as

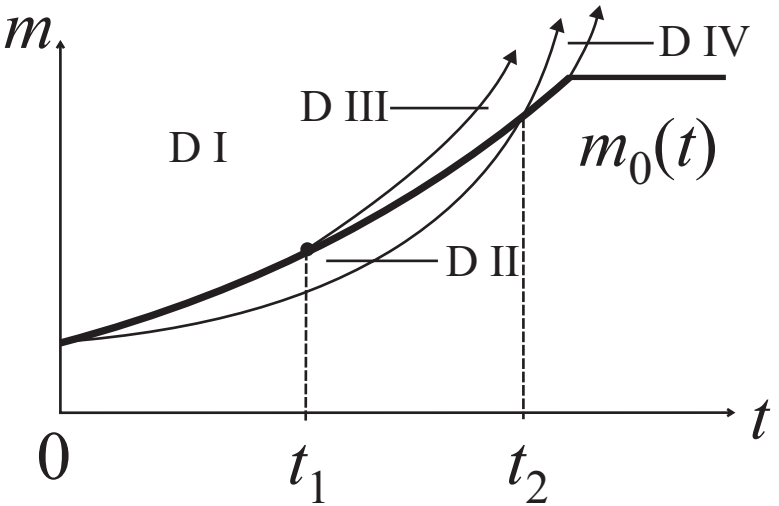

Figure 4.10 A plot of the birth mass $m_0(t)$, indicated by the thick curve, together with three growth curves, thin curves, for a situation in which the mass at birth increases faster with time than the cell mass of the smallest cells. The single-cell growth rate becomes less than the rate of increase in newborn cell mass at time 0. At this point in time, a domain starts to form below the curve for the birth mass $m_0(t)$. Initially, the growth flux of cells is in a direction from the domain above $m_0(t)$ into the domain below $m_0(t)$. At time t_1, the rate of single-cell mass growth becomes equal to the rate of increase in the mass of newborn cells and the cell growth flux changes direction so it is from the domain below $m_0(t)$ to the domian above $m_0(t)$. Finally, at t_2, the smallest cell in the culture attains a mass equal to that of newborn cells and the region below $m_0(t)$ vanishes.

$$m_0(t) = \begin{cases} e^{\nu_1} - 1, & t < 0 \\ e^{\nu_1 + t(\nu_2 - \nu_1)} - 1, & 0 < t < 1 \\ e^{\nu_2} - 1, & t > 1 \end{cases}$$

When this function is plotted together with the growth curve of the smallest cell at time zero, the picture in Fig. 4.10 emerges. Study this figure carefully!

One of the domain boundaries is $m_0(t)$, the mass of newborn cells. However, initially, the smallest cells in the population grow slower than $m_0(t)$ and therefore their growth curves must lie below $m_0(t)$, forming a separate domain, here called D II. This situation persists until time t_1, at which the growth rate of the smallest cells equals the rate of change in $m_0(t)$, when the term $(\nu(t)\, m_0(t) - dm_0/dt)$ in Eq. (4.10) equals zero. For $t < t_1$, the flux out of the birth control point is negative, for $t > t_1$ it is positive, and for $t < t_1$, the cell balance at birth is an initial condition for the solution in D II, while for $t > t_1$ it is an initial condition for the solution in domain D III. Because of

this change in the structure of the solution, a domain boundary is formed by the growth curve that starts at the point $(t_1, m_0(t_1))$.

The domains that are formed can now be described in words as follows: Domain D I represents cells larger than $m_0(t)$ and larger than the cells on the growth curve starting at t_1, $m_0(t_1)$. The initial condition for D I is the initial distribution. D II is the cells that are formed below $m_0(t)$ while the flux out of this control point is negative. The initial condition for D II is a cell balance at $m_0(t)$ between $t = 0$ and $t = t_1$. D III is the cells on the growth curves intersecting $m_0(t)$ while the flux out of this control point is positive. D III is bounded on the left by the domain boundary that emanates from $(t_1, m_0(t_1))$, below by $m_0(t)$, and on the right by the domain boundary emanating from $(t_2, m_0(t_2))$, where t_2 is the time at which domain D II vanishes. The initial condition is a cell balance along $m_0(t)$ for $t_1 < t < t_2$. This cell balance accounts for both the growth flux from below, from D II, and the birth flux. Finally, D IV represents the cells born after t_2, when there is no longer a growth flux into the birth control point.

The time t_1 is defined as the time when the rate of cell mass increase becomes equal to the rate of increase in newborn cell mass, so this time is found by solving

$$\frac{dm_0(t)}{dt} = r(m_0) \quad \Rightarrow$$

$$(\nu_2 - \nu_1)\, e^{\nu_1 + t_1(\nu_2 - \nu_1)} = \nu_2(e^{\nu_1 + t_1(\nu_2 - \nu_1)} - 1) \quad \Rightarrow$$

$$t_1 = \frac{1}{\nu_2 - \nu_1} \ln\left(\frac{\nu_2}{\nu_1 e^{\nu_1}}\right) \approx 0.4327906$$

The time at which the domain vanishes, t_2, is found by determining the intersection of $m_0(t)$ with the growth curve of the smallest cell in the culture. Assuming first that this point occurs before time 1, the equation for t_2 becomes

$$(e^{\nu_1} - 1)e^{\nu_2 t_2} = e^{\nu_1 + t_2\,(\nu_2 - \nu_1)} - 1$$

which must be solved numerically, giving a root less than 1 as assumed:

$$t_2 \approx 0.924825$$

In Fig. 4.11, more of the initial domains for our problem are plotted to scale. The figure was obtained by placing a figure of the mother cell domains above a figure of the daughter cell domains. The horizontal line that runs through the figure one-third up from the bottom therefore represents the cell state of newborn mother cells or of daughter cells that have reached the cell mass m^*.

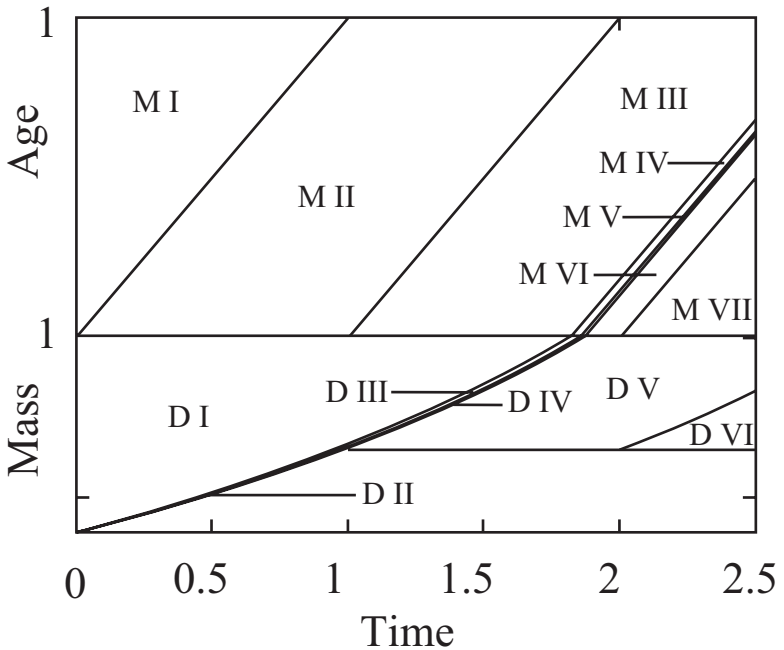

Figure 4.11 Solution domains in a shift up in single cell specific growth rate of budding yeast. The bottom one-third of the diagram represents the domains for daughter cells and the top two-thirds the domains for mother cells. The state of daughter cells is specified by cell mass, while the state of mother cells is indicated by cell age.

The domain structure is complex, and there is no simple repetitive structure appearing at larger times. A simple repetitive structure does not develop because the duration of the daughter part of the cell cycle is not a simple fraction or multiple of the duration of the mother cell cycle. The two cycles are not close to being synchronized. However, for special cases, such as if the duration of the daughter cell cycle exactly equals the duration of the mother cell cycle, a very simple repetitive domain structure does appear. (Convince yourself of this by drawing the relevant diagrams.) Domains D II, D III, and D IV are so narrow that they are virtually obscured by the lines that indicate domain boundaries. It is tempting to ignore the solutions in these domains, as they appear so insignificant, but the small size does not mean that they are insignificant. All daughter cells born between time 0 and 1 pass through domains D III and D IV and, as these domains are so narrow, these cells experience a significant contraction of state space. The value of the frequency function must therefore be large in these domains. Thus, a small and seemingly insignificant region of state space may still contain a significant fraction of the total number of cells in a population.

Now that the domains have been carefully defined, we can find the transient distribution of states. We will seek the solution only up to time 1, and

we will therefore only need the solutions in domains M I, M II, D I, D II, D III, and D IV.

Domain M I Straightforward:

$$W_{\mathrm{MI}}(t, a) = W_{\mathrm{M0}}(a - t)$$

Domain D I Equally straightforward:

$$W_{\mathrm{DI}}(t, m) = W_{\mathrm{D0}}(me^{-\nu_2 t})e^{-\nu_2 t}$$

Notice the factor $e^{-\nu_2 t}$ in the solution in domain D I. This term is caused by the fact that the single-cell growth curves diverge and the state space therefore expands. Thus the frequency must decrease, which is taken care of by the term $e^{-\nu_2 t}$.

Domain M II In this domain, the boundary condition along the initial manifold is given by the cell balance

$$W_{\mathrm{MII}}(t, 0) = W_{\mathrm{MI}}(t, 1) + W_{\mathrm{DI}}(t, 1)\nu_2, \qquad 0 < t < 1$$

which can be written in terms of the two initial distributions as

$$W_{\mathrm{MII}}(t, 0) = W_{\mathrm{M0}}(1 - t) + W_{\mathrm{D0}}(e^{-\nu_2 t})e^{-\nu_2 t}\nu_2$$

and the solution becomes

$$W_{\mathrm{MII}}(t, a) = W_{\mathrm{M0}}(1 - t + a) + e^{-\nu_2(t-a)}W_{\mathrm{D0}}(e^{-\nu_2(t-a)})\nu_2$$

Domain D II For this domain, the initial manifold is the section of the curve $m_0(t)$ that lies between time 0 and t_1. Let us first write the cell balance along this curve segment. The cell flux is from domain D I, the older cells, to domain D II, the younger cells, and the flux into the control point $m_0(t)$ is the growth flux from D I, so

$$\text{Cell flux into } m_0(t) = W_{\mathrm{DI}}(t, m_0(t))\left(\frac{dm_0}{dt} - \nu_2 m_0(t)\right)$$

where the two terms inside the parentheses have been switched relative to their position in Eq. (4.10) in order to give a positive flux. Similarly, the flux out of the control point is the growth flux into D II:

$$\text{Cell flux out of } m_0(t) = W_{\mathrm{DII}}(t, m_0(t))\left(\frac{dm_0}{dt} - \nu_2 m_0(t)\right)$$

and the cell balance becomes

$$W_{\mathrm{DII}}(t, m_0(t))\left(\frac{dm_0}{dt} - \nu_2 m_0(t)\right) = W_{\mathrm{DI}}(t, m_0(t))\left(\frac{dm_0}{dt} - \nu_2 m_0(t)\right) + W_{\mathrm{MI}}(t, 1)$$

or

$$W_{\mathrm{DII}}(t, m_0(t)) = \frac{W_{\mathrm{MI}}(t, 1)}{\dfrac{dm_0}{dt} - \nu_2 m_0(t)} + W_{\mathrm{DI}}(t, m_0(t))$$

$$= \frac{W_{\mathrm{M0}}(1-t)}{1 - \nu_1 e^{\nu_1(1-t) + \nu_2 t}} + e^{-\nu_2 t} W_{\mathrm{D0}}\left(e^{\nu_1(1-t)} - e^{-\nu_2 t}\right), \quad 0 < t < t_1$$

Now parameterize the initial manifold, the $m_0(t)$ curve, as follows:

$$t = \tau, \quad m = e^{\nu_1(1-\tau) + \nu_2 \tau} - 1, \quad W_{\mathrm{DII}}(\tau) = W_{\mathrm{DII}}(\tau, m_0(\tau))$$

Solve the characteristic base equations and invert the results to obtain τ and s:

$$\left.\begin{aligned} &\frac{dm}{ds} = \nu_2 m, \qquad m(0) = e^{\nu_1(1-\tau) + \nu_2 \tau} \Rightarrow m(s, \tau) = e^{\nu_1(1-\tau) + \nu_2(\tau + s)} \\ &\frac{dt}{ds} = 1, \qquad t(0) = \tau \Rightarrow t = s + \tau \end{aligned}\right\} \Rightarrow$$

$$s = t - 1 + \frac{\ln m - \nu_2 t}{\nu_1}$$

$$\tau = 1 - \frac{\ln m - \nu_2 t}{\nu_1}$$

Solving the last characteristic equation gives

$$\frac{dW_{\mathrm{DII}}}{ds} = -\nu_2 W_{\mathrm{DII}}, \; W_{\mathrm{DII}}(0) = W_{\mathrm{DII}}(\tau, m_0(\tau)) \Rightarrow$$

$$W_{\mathrm{DII}}(s, \tau) = e^{-\nu_2 s} W_{\mathrm{DII}}(\tau, m_0(\tau))$$

Substituting in the expressions for τ and s gives

$$W_{\mathrm{DII}}(t, m) = e^{-\nu_2(t - 1 + (\ln m - \nu_2 t)/\nu_1)} \left[\frac{W_{\mathrm{M0}}\left(\dfrac{\ln m - \nu_2 t}{\nu_1} \right)}{1 - \nu_1 m e^{-\nu_2 t + \nu_2 (1 - (\ln m - \nu_2 t)/\nu_1)}} \right.$$

$$\left. + e^{-\nu_2 (1 - (\ln m - \nu_2 t)/\nu_1)} W_{\mathrm{D0}}\left(m e^{-\nu_2 t} - e^{-\nu_2 (1 - (\ln m - \nu_2 t)/\nu_1)} \right) \right]$$

Domain D III In this domain, the initial manifold is the section of the curve $m_0(t)$ that lies between t_1 and t_2. Along this section of the curve, the cell flux is in the usual direction, from the domain with the younger cells, D II, to the domain with the older cells, D III. The growth flux in the cell balance is therefore written the usual way, and the cell balance becomes

$$W_{\mathrm{DIII}}(t, m_0(t)) = \frac{W_{\mathrm{MI}}(t, 1)}{\nu_2 m_0(t) - \dfrac{dm_0}{dt}} + W_{\mathrm{DII}}(t, m_0(t))$$

The characteristic equations and the parameterization of the initial manifold is identical to domain II, and we can therefore take the results from there and proceed straight to the substitution of the balance equation into the solution for $W_{\mathrm{DIII}}(s, \tau)$. Further substituting the expressions for s and τ gives

$$W_{\mathrm{DIII}}(t, m) = e^{-\nu_2(t - 1 + (\ln m - \nu_2 t)/\nu_1)} \left[\frac{W_{\mathrm{M0}}\left(\dfrac{\ln m - \nu_2 t}{\nu_1} \right)}{1 - \nu_1 m e^{-\nu_2 t + \nu_2 (1 - (\ln m - \nu_2 t)/\nu_1)}} \right.$$

$$+ \exp\left\{ \frac{\nu_2}{\nu_1} \left(\ln m - \nu_2 t - \ln\left(m e^{-\nu_2 t + \nu_2 (1 - (\ln m - \nu_2 t)/\nu_1)} - 1 \right) \right. \right.$$

$$\left. \left. + \nu_2 \left(1 - \frac{\ln m - \nu_2 t}{\nu_1} \right) \right) \right\}$$

$$\cdot \left\{ \frac{W_{\mathrm{M0}}\left(\dfrac{\ln m - \nu_2 t}{\nu_1} \right)}{1 - \nu_1 m e^{-\nu_2 t + \nu_2 (1 - (\ln m - \nu_2 t)/\nu_1)}} \right.$$

$$\left. \left. + e^{-\nu_2 (1 - (\ln m - \nu_2 t)/\nu_1)} W_{\mathrm{D0}}\left(m e^{-\nu_2 t} - e^{-\nu_2 (1 - (\ln m - \nu_2 t)/\nu_1)} \right) \right\} \right]$$

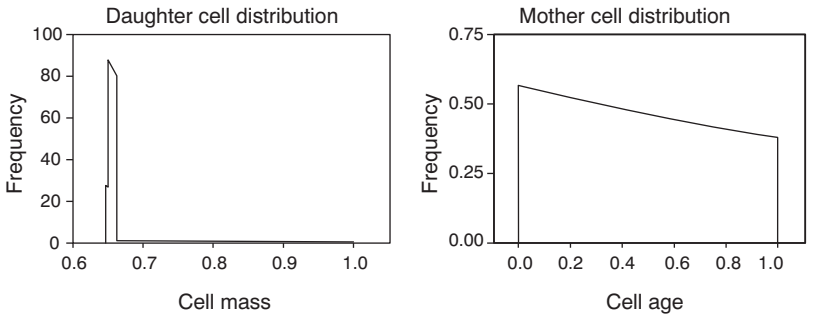

Figure 4.12 Daughter cell mass distribution (left plot) and mother cell age distribution (right plot) following a shift up in cell mass growth rate.

Domain D IV The initial manifold is the section of $m_0(t)$ between t_2 and 1. There is no domain below this manifold, so the cell balance is quite simple.

$$W_{\mathrm{DIV}}(t,\, m_0(t)) = \frac{W_{\mathrm{MI}}(t,\, 1)}{\nu_2 m_0(t) - \dfrac{dm_0}{dt}}, \qquad t_2 < t < 1$$

and the characteristic equations are the same as for the previous domains. The following solution is now obtained:

$$W_{\mathrm{DIV}}(s,\ \tau) = e^{-\nu_2 s}\, \frac{W_{\mathrm{M0}}(1-\tau)}{1 - \nu_1 e^{\nu_1 (1-\tau) + \nu_2 \tau}}$$

and, from the previously obtained expression for s and τ, this becomes

$$W_{\mathrm{DIV}}(t,\, m) = e^{-\nu_2 (t - 1 + (\ln m - \nu_2 t)/\nu_1)}\, \frac{W_{\mathrm{M0}}\left(\dfrac{\ln m - \nu_2 t}{\nu_1}\right)}{1 - \nu_1 m e^{-\nu_2 (t - 1 + (\ln m - \nu_2 t)/\nu_1)}}$$

The two distributions at time 1 are plotted in Fig. 4.12 for the distribution $W_{\mathrm{M0}} = \nu_1 e^{-\nu_1 a}$ and $W_{\mathrm{D0}} = (1 - e^{-\nu_1})/m^2$ as the initial condition.

All daughter cells that were born between times 0 and 1 were born close to the state of the smallest cell in the culture. Consequently, all these cells are close to each other in state space and therefore form a sharp peak in the daughter cell distribution. The value of the distribution in the peak is so large that the remaining parts of the distribution are not even visible in a linear plot such as this. The shoulder visible on the left side of the peak is not an artifact of the plotting routine. It represents the value of the solution in domain D IV, which is less than the value in domain D III, the domain that forms the bulk of the peak.

4.5 Solutions for Large Times

In many transient calculations, one either undertakes to find the distribution of states as they approach steady state under conditions when the control points are fixed, or the dynamic calculations get to a point where this is the case. Once the transient in the control point dynamics has died out, the tedious domain-by-domain solution procedure can be abolished in favor of a more powerful method capable of providing a solution for arbitrary large times. The method can be used not just for situations in which the control points have become fixed but in any case where the control point dynamics has settled down to a simple repetitive behavior such as periodically changing control points.

A simple recursion formula between solutions at different times can often be obtained quite easily by perceptive guessing. The guessing is done after studying the solution for the first couple of domains after the control points have become fixed. For instance, for a chemostat with a binary fission organism that divides at the age a_d, it is trivial to show that if the age distribution at time t_0 is $W_0(a)$, then the age distribution at time $t_0 + a_d$ is $2\,W_0(a)\,e^{-Dt}$. But the origin of the time axis is arbitrary, so one can certainly generalize this result to the following recursion formula:

$$W(t_0 + na_d,\, a) = 2W(t_0 + (n-1)a_d,\, a)e^{-Da_d}$$

or, written more compactly in a notation we will use in the following paragraphs,

$$W_n(a) = 2W_{n-1}(a)e^{-Da_d}$$

where the subscript n indicates n time steps of length a_d. When one looks at this result, the following general solution suggests itself:

$$W_n(a) = 2^n\ W_0(a)\ e^{-Da_d n}$$

This solution may seem obvious, but this is only because the problem is so simple. In more complex cases, it will be harder to infer a general solution from a recursion formula, and the proposed general solution should always be shown to be correct by an induction proof.

Proof by induction requires two steps: The first step is a proof that the induction hypothesis, the proposed general solution, is true for some low value of n, usually 0 or 1. The second step is a proof showing that if the induction hypothesis is true for n, then it must also be true also for $n + 1$. In our case, the proposed solution is trivially true

for $n = 0$. If the solution is assumed true for n, then we can use $W_n(a)$ as the initial condition for a transient calculation and we find that the age distribution at time a_d or $W_{n+1}(a)$ is $2e^{-Da}dW_n(a)$ or $2^{n+1}e^{-Da}d^{(n+1)}W_0(a)$. But this is the induction hypothesis evaluated at $n + 1$, and the proof is complete.

Example 4.8: Recursion formula for budding yeast In the previous example we calculated the transient following a shift up in specific cell mass growth rate for budding yeast. The calculations generated a plethora of domains, but this fact becomes irrelevant as soon as the control point dynamics has died out at the time $t = 1$. The solution at that point in time becomes the initial condition for the recursion formula, and to obtain this formula, we need only the solution at some time later than the initial time. We therefore need solutions in only a few domains, exactly enough to find the distribution of states at some later time, and the domains should be those that are naturally generated in a transient calculation with fixed control points.

We will take the solution from the previous example at $t = 1$ as the initial condition for a chemostat with dilution rate and single-cell specific growth rate equal (which will assure the existence of a steady state) and set $D = \nu = \nu_2 = \frac{1}{2}$.

The model equations, in dimensionless state variables, are the population balance equations for mother and daughter cells,

$$\frac{\partial W_M}{\partial t} + \frac{\partial W_M}{\partial a} = -\nu W_M$$

$$\frac{\partial W_D}{\partial t} + \frac{\partial \nu m W_D}{\partial m} = -\nu W_D$$

the cell balances

$$W_M(t, 0) = W_M(t, 1) + \nu W_D(t, 1)$$

$$(e^{\nu} - 1)\nu\ W_D(t, e^{\nu} - 1) = W_M(t, 1)$$

and the initial conditions

$$W_M(0, a) = W_{M0}(a), \quad W_D(0, m) = W_{D0}(m)$$

The initial domains in which we will seek a solution are shown in Fig. 4.13.

The solution procedure should be well established by now, so we will not show any intermediate steps, only the final domain solutions in the order in which they are obtained. (For the reader not yet comfortable with solving transient problems, checking the results below is an excellent exercise.)

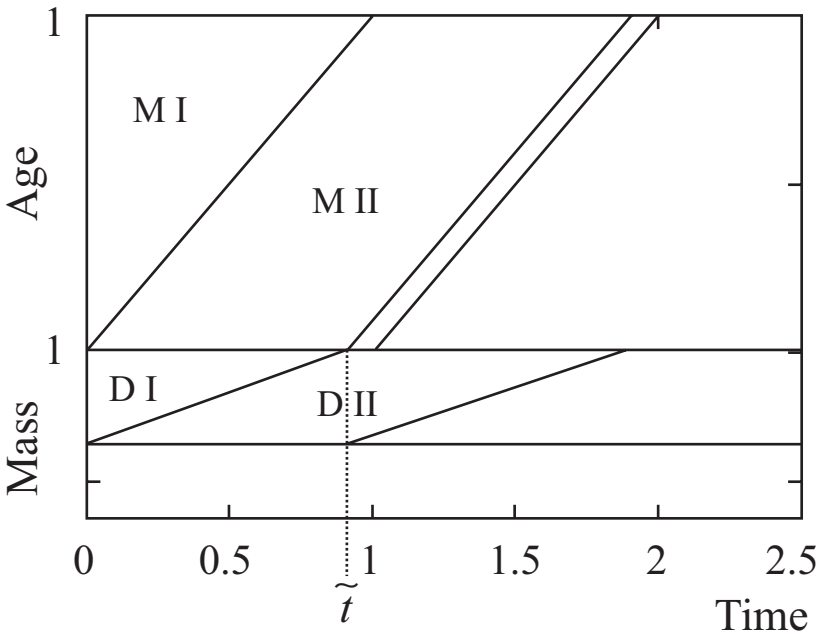

Figure 4.13 Initial domains used in the calculation of a recursion formula for budding yeast transients.

Domain M I $0 < t < 1, t < a < 1$

$$W_{\mathrm{MI}}(t, a) = W_{\mathrm{M0}}(a - t)e^{-\nu t}$$

Domain D I $0 < t < -\dfrac{[\ln(e^{\nu} - 1)]}{\nu}, \quad (e^{\nu} - 1)e^{\nu t} < m < 1$

$$W_{\mathrm{DI}}(t, m) = W_{\mathrm{D0}}(me^{-\nu t})e^{-2\nu t}$$

Domain M II

$$W_{\mathrm{MII}}(t, a) = W_{\mathrm{M0}}(1 - t + a)e^{-\nu t} + \nu W_{\mathrm{D0}}(e^{-\nu(t-a)})e^{-2\nu t}e^{\nu a}$$

Domain D II

$$W_{\mathrm{DII}}(t, m) = W_{\mathrm{M0}}\left(1 - t + \frac{\ln\left(\dfrac{m}{e^{\nu} - 1}\right)}{\nu}\right)\frac{e^{-\nu t}}{\nu m}$$

The largest time at which a solution for the distribution of states can be found from these domain solutions is at $\tilde{t} = -\ln(e^{\nu} - 1)\big/\nu \approx 0.8655045$.

$$W_{\mathrm{M}}(\tilde{t}, a) = \begin{cases} W_{\mathrm{M0}}(1 - \tilde{t} + a)(e^{\nu} - 1) \\ \quad + \nu W_{\mathrm{D0}}(e^{\nu a}(e^{\nu} - 1))e^{\nu a}(e^{\nu} - 1)^2, & 0 < a < \tilde{t} \\ W_{\mathrm{M0}}(a - \tilde{t})(e^{\nu} - 1), & \tilde{t} < a < 1 \end{cases}$$

$$W_{\mathrm{D}}(\tilde{t}, m) = W_{\mathrm{M0}}\left(1 + \frac{\ln m}{\nu}\right)\frac{\left(e^{\nu} - 1\right)}{\nu m}, \qquad e^{\nu} < m < 1$$

so the time step in the recursion formula will be equal to $\tilde{t}$ and the recursion formula can be stated as

$$W_{\mathrm{M},n}(a) = \begin{cases} W_{\mathrm{M},n-1}(1 - \tilde{t} + a)(e^{\nu} - 1) + \\ \quad \nu W_{\mathrm{D},n-1}(e^{\nu a}(e^{\nu} - 1))(e^{\nu} - 1)^2 e^{\nu a}, & 0 < a < \tilde{t} \\ W_{\mathrm{M},n}(a - \tilde{t})(e^{\nu} - 1), & \tilde{t} < a < 1 \end{cases}$$

$$W_{\mathrm{D},n}(m) = W_{\mathrm{M},n-1}\left(1 + \frac{\ln m}{\nu}\right)\frac{\left(e^{\nu} - 1\right)}{m\nu}, \qquad e^{\nu} < m < 1$$

This result has been used to calculate and plot the distributions of states at large times after a shift up in specific growth rate (Fig. 4.14). The initial condition for the recursion formula is the distribution found in the previous example at the dimensionless time 1, the time at which the control points cease to move. This initial distribution is plotted in Fig. 4.12. The solution at the late time shows that the peak of cells that was generated by the step-up has multiplied into numerous peaks. This happens because, when the cells in the peak divide, two new peaks are formed, a daughter cell peak and a mother cell peak; these new peaks in turn double when the cells in them divide. The peak number will therefore increase exponentially with time until the peaks become so numerous that they start to become superimposed on one another.

The recursion formula result is quite complex and it is far from easy to guess a general solution for W_n. However, the recursion formula is still very useful because it provides a practical tool for rapid calculation of the distribution at any reasonable time. Some software packages such as Maple and Mathematica allow the user to define functions recursively, in which case calculation of the distribution is straightforward. However, it is obvious from the recursion formula that, as n increases, the number of function evaluations increases rapidly, roughly as the exponential of n, and the computational time therefore places a practical limit of the values of n for which the recursion formula is useful.

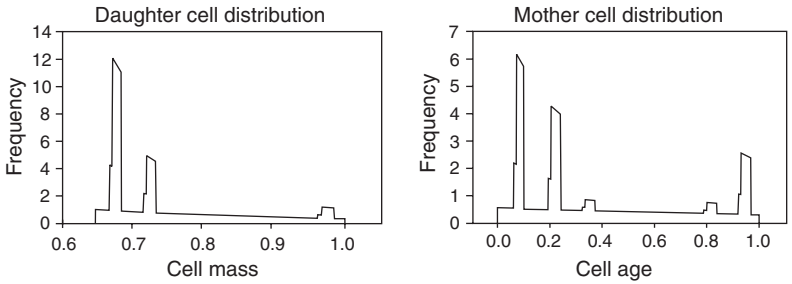

Figure 4.14 The distributions of states for a budding yeast 10 time steps of length $\tilde{t}$ after the control points have become fixed.

Alternatively, the initial distribution of states can be approximated by a discrete distribution and stored in an array or table. Distributions at later times are calculated from the recursion formula and stored in additional tables, one new table for each time step forward. However, calculation of the entries in a new table requires interpolation in the table representing the previous time. Each interpolation introduces an error, which is particularly large when the interpolation is between two points that span a discontinuity in the distribution. This tabular method therefore results in a degradation of the accuracy of the table entries at larger times, and an increasingly fine discretization of the distributions is needed for longer times. Of course, a finer discretization gives rise to a larger computational time, making this method unsuitable for finding the distributions during long transients.

A comparison of the exact use of the recursion formula and a calculation using tabular interpolation is shown in Fig. 4.15. The figure shows the mother cell distribution after 19 time steps. The degradation of the values obtained by the tabular interpolation method, the dots, is becoming visible around the discontinuities. However, calculation of the distribution using the exact recursion formula required approximately 40 times as much computer time as the calculation using tabular interpolation. The computational time required for the exact recursion formula increases approximately exponentially with the number of recursion steps n, as $e^{0.4\,n}$, while the computational time for the tabular interpolation is roughly proportional to n. However, the proportionality constant increases with the number of entries used in the tables.

In the previous example, the recursion formula was so complex that guessing a general solution was difficult, and readers will probably agree that it would be nice to have a systematic method for finding a closed-form solution at large times. A systematic solution method does exist and is based on the observation that, when the control points are stationary, the tiling of the state space by the solution domains almost always becomes periodic at some point in time [40, 44]. This is seen most easily in the case of the age distribution, which is the only case that will be covered here. Consider the situation depicted in Fig. 4.16, in which

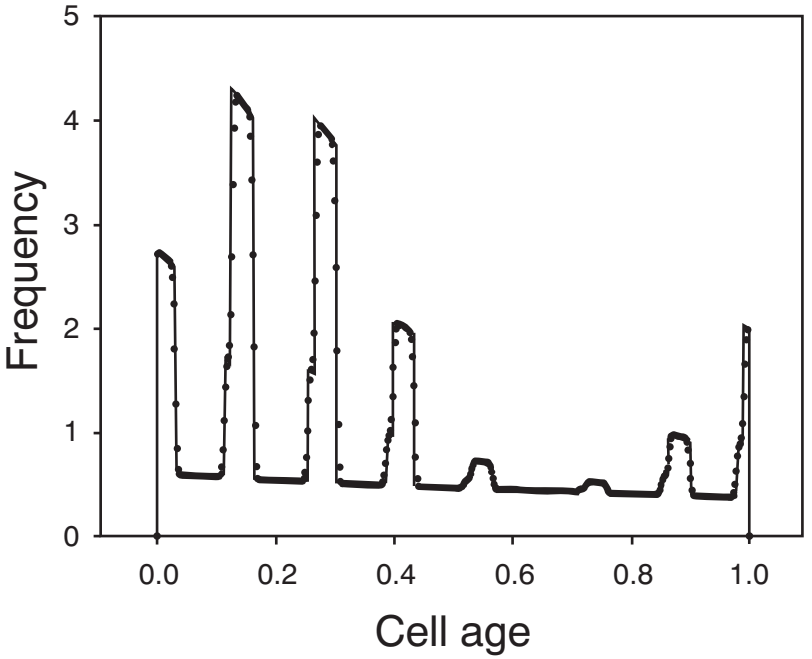

Figure 4.15 The distribution of mother cell ages for budding yeast by 19 time steps of length τ after the control points have become fixed. The solid line is obtained using the recursion formula, and 500 points are plotted. The dots represent the result obtained by tabular interpolation using 500 entries in the table.

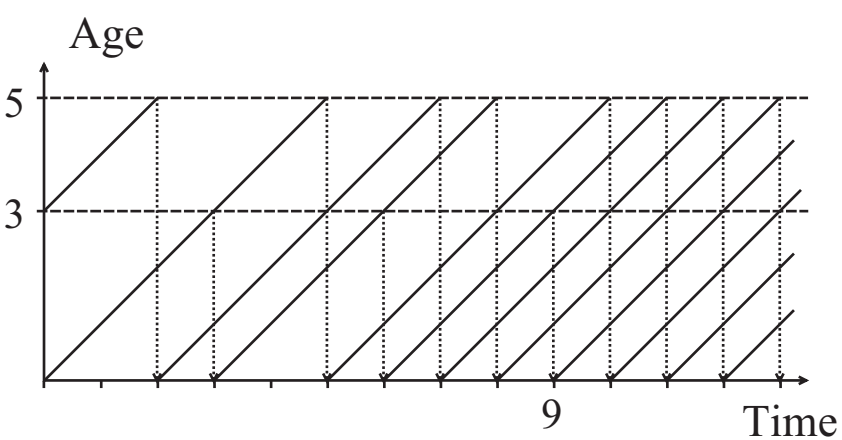

Figure 4.16 Initial solution domains obtained with control points at ages 3 and 5. A repetitive structure arises after time 9. The vertical arrows indicate points where a domain boundary bifurcates to create two new boundaries. One, a straight line continuation of the old boundary represents cells that have not divided; another, starting at age zero, represents cells that have divided.

control points are located at ages 3 and 5. Some of the cells divide at age 3, the remaining cells at age 5. Thus, the domain boundaries that intersect the horizontal line at the division age 3 bifurcate and form two new domain boundaries: one corresponding to the cells that did not divide and one to the cells that did divide. As a result, the number of domain boundaries increases with time until, at a time equal to 9 in this case, the system has reached a state where bifurcating domain boundaries form on already-existing boundaries and no new boundaries therefore form. After this time, the tiling of the state space is periodic or repetitive in the sense that the geometry of the solution domains between t and $t + 1$ is identical to the geometry between $t + N$ and $t + N + 1$ (N integer).

A little contemplation will show that this repetitive structure will arise when the ratio of the distance between any two control points is a rational number. In this case, the tiling will eventually repeat with a period equal to the largest number equal to an integer fraction of all the age differences between control points.

Because of this repetitive structure of the domain tiling, the distribution of states can, at any time, be conveniently partitioned into a set of solutions, each valid over a subinterval of the age axis between two adjacent domain boundaries. This is quite similar to the situation encountered in solving for the steady-state distribution in control point models. The difference is only that, in the transient case, the evolution of the domain boundaries partitions the age space into finer intervals than those defined by the control points.

Let the vertical distance or age difference between two adjacent domain boundaries in this repetitive structure be Δ. Clearly, Δ is also the period with which the tiling repeats. Assume that the age distribution is known for $t = n\ \Delta$, where n is an integer. The domain structure depicted in the left diagram of Fig 4.17 now appears. Before finding the solutions in these domains, we will introduce the simplified nomenclature, indicated in the figure, that $W_m(a,\ n\ \Delta)$ is the age distribution in the subinterval between age $(m\ -\ 1)\ \Delta$ and $m\ \Delta$ at time $n\ \Delta$.

It turns out that using the physical age of a cell as a state parameter in the calculations that follow results in rather cumbersome expressions. It is more convenient to work with a state parameter, call it $\hat{a}$, which runs between 0 and Δ for each subinterval. Thus, let the state parameter for W_m be $\hat{a} = a - (m - 1)\Delta$. This variable transformation is simply a translation along the age axis, and it does not alter the form of the age population balance equation itself. The diagram on the right of Fig. 4.17, showing the domains relevant to obtaining the solution for $W_m(\hat{a}, t)$, now results. Notice that when the translated age $\hat{a}$ is used as

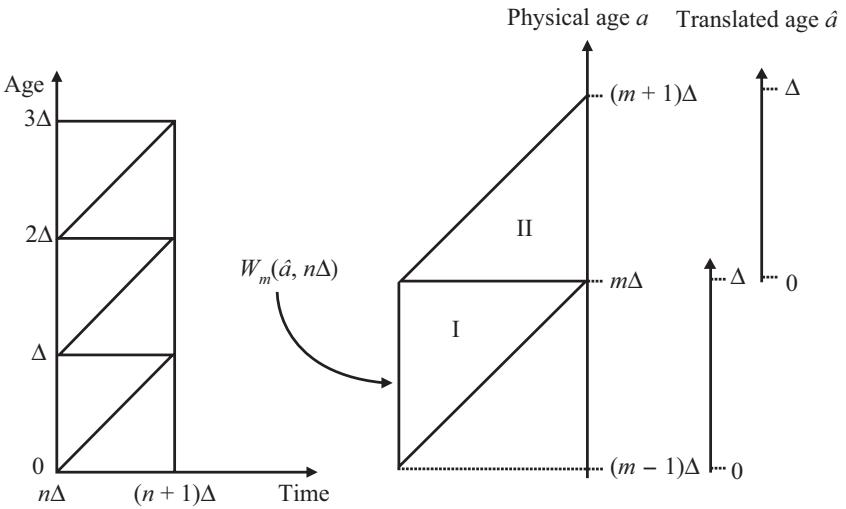

Figure 4.17 Solution domains between time $n\Delta$ and $(n + 1)\Delta$ in the repetitive domain structure with period Δ.

a state coordinate, the diagram is identical for all values of m with an age parameter that runs between zero and Δ, and the problem that must be solved is thus the same for all values of m. The solutions in domains I and II are as follows.

In domain I, the initial condition is

$$t = n\Delta \Rightarrow W_{\mathrm{I}}(\hat{a},\ n\Delta) = W_m(\hat{a},\ n\Delta)$$

and the solution is trivial:

$$W_{\mathrm{I}}(\hat{a},\ t) = W_m(\hat{a} - (t - n\,\Delta),\ n\,\Delta)e^{-D(t - n\,\Delta)} \tag{4.11}$$

The domain boundary between domains I and II may be a control point, and some fraction of the cells may therefore divide at this point. Let the fraction of cells that divide be Φ_m. The initial condition for domain II becomes

$$a = 0 \Rightarrow W_{\mathrm{II}}(0,\ t) = (1 - \Phi_m)W_{\mathrm{I}}(\Delta, t)$$

and the solution in domain II then is

$$\begin{aligned} W_{\mathrm{II}}(\hat{a},\ t) &= W_{\mathrm{II}}(0,\ t - \hat{a})e^{-D\hat{a}} \\ &= (1 - \Phi_m)W_m((n + 1)\Delta - t + \hat{a},\ n\,\Delta)e^{-D(t - n\,\Delta)} \end{aligned}$$

But, since $W_{m+1}(\hat{a}, (n+1)\Delta)$ is this solution evaluated at $t = (n+1)\Delta$, one obtains the recursion formula

$$W_{m+1}(\hat{a},\ (n+1)\Delta) = (1 - \Phi_m)W_m(\hat{a},\ n\Delta)e^{-D\Delta},\quad m > 1$$

The recursion formula for W_1 is a special case and requires the use of the renewal equation. Let the solution in the domain along $a = 0$ be $W_{\mathrm{I}}(a,\ t)$. Then, from the renewal equation and using the result in Eq. (4.11), one obtains

$$\begin{aligned} W_{\mathrm{I}}(0,\ t) &= 2\sum_{m=1}^{M}\Phi_m W_{\mathrm{I}}(\Delta, t) \\ &= 2\sum_{m=1}^{M}\Phi_m W_m((n+1)\Delta - t,\ n\Delta)e^{-D(t-n\Delta)} \Rightarrow \\ W_{\mathrm{I}}(\hat{a},\ t) &= 2\sum_{m=1}^{M}\Phi_m W_m((n+1)\Delta - (t-\hat{a}),\ n\Delta)e^{-D((t-\hat{a})-n\Delta)}e^{-D\hat{a}} \end{aligned}$$

and $W_1(\hat{a}, (n+1)\Delta)$ is this solution evaluated at $t = (n+1)\Delta$.

$$W_1(\hat{a},\ (n+1)\Delta) = 2\sum_{m=1}^{M}\Phi_m W_m(\hat{a},\ n\Delta)e^{-D\Delta}$$

The results are most conveniently written in vector-matrix notation as

$$\overline{W}_{n+1} = A\overline{W}_n \tag{4.12}$$

where

$$\overline{W}_n = \begin{pmatrix} W_1(\hat{a},\ n\Delta) \\ W_2(\hat{a},\ n\Delta) \\ W_3(\hat{a},\ n\Delta) \\ \vdots \\ W_M(\hat{a},\ n\Delta) \end{pmatrix}$$

and

$$A = \begin{pmatrix} 2\Phi_1 e^{-D\Delta} & 2\Phi_2 e^{-D\Delta} & \cdots & 2\Phi_{M-1} e^{-D\Delta} & 2\Phi_M e^{-D\Delta} \\ (1-\Phi_1)e^{-D\Delta} & 0 & \cdots & 0 & 0 \\ 0 & (1-\Phi_2)e^{-D\Delta} & \cdots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \cdots & (1-\Phi_{M-1})e^{-D\Delta} & 0 \end{pmatrix}$$

Since cells that grow to attain the highest possible age must all divide at this age, Φ_M must equal unity.

At steady state, a linkage equation must exist, relating the dilution rate to the many single-cell parameters of the model. This equation is obtained as follows. Note that, at steady state, $\overline{W}_{n+1} = \overline{W}_n$. It follows from the first of the vector-matrix equations that

$$W_1 = 2e^{-D\Delta} \sum_{m=1}^{M} \Phi_m W_m$$

and the remaining equations give

$$W_m = (1-\Phi_{m-1})e^{-D\Delta} W_{m-1}$$

which, when used recursively, gives

$$W_m = \prod_{j=1}^{m-1} (1-\Phi_j) e^{-D\Delta(m-1)} W_1$$

Putting both results together and dividing out W_1 gives the linkage equation

$$\frac{1}{2} = \sum_{m=1}^{M} \left(\prod_{j=1}^{m-1} (1-\Phi_j) \right) \Phi_m (e^{-D\Delta})^m \tag{4.13}$$

Notice that it is a polynomial equation in $e^{-D\Delta}$. As there are many powerful numerical tools for finding polynomial roots, it is a trivial matter to find the appropriate steady-state value of the dilution rate for a given model.

Having derived Eq. (4.12), which is a linear, first-order, finite-difference equation with constant coefficients, we are essentially done, since this equation represents a well-understood mathematical problem for which a solution method exists. Of course, going through the trouble of finding the analytical solution may not always be worthwhile because Eq. (4.12) can be used to easily compute consecutive values of W_n from any given initial condition. However, it is quite informative to study the analytical solution because it provides added insight into the structure and properties of the transient dynamics. The analytical solution method is therefore outlined briefly below, and understanding it does require some background knowledge of linear algebra, in particular about the algebraic eigenvalue problem. This subject matter is described in most textbooks on linear algebra.

If the eigenvalues of the matrix A are all simple or semisimple, then there exists a so-called similarity transformation of the form

$$A = K\Lambda K^{-1}$$

where K is a matrix of eigenvectors and Λ is a diagonal matrix of eigenvalues. Substitution into Eq. (4.12) yields

$$\overline{W}_{n+1} = K \Lambda K^{-1}\overline{W}_n \Rightarrow K^{-1}\overline{W}_{n+1} = \Lambda K^{-1}\overline{W}_n \Rightarrow Q_{n+1} = \Lambda Q_n$$

Since Λ is a diagonal matrix, the equations in the last expression are uncoupled with each equation of the form

$$q_{m,\,n+1} = \lambda_m q_{m,\,n} \Rightarrow q_{m,\,n} = \lambda_m^n q_{m,\,0}$$

where we can assume that all the $q_{m,0}$ are known from the initial condition. The solution in terms of Q is backtransformed to get W:

$$\overline{W}_n = KQ_n$$

Notice that, if all the eigenvalues are located inside the unit circle in the complex plane, then all the components of the solution vector will go to zero as n goes to infinity. In other words, the sterile or cell-free solution is stable. If just one of the eigenvalues is outside the unit circle, then the solution will increase without bounds and it is therefore unstable. This result, stability only when the eigenvalues are inside the unit circle, is different from the well-known result in continuous

time models where stability requires the eigenvalues to be in the left half-plane of the complex plane. The most interesting situation is when a nonsterile steady state exists. This is expected to be the case when Eq. (4.13) holds. In this case, the limiting behavior at large n is expected to be constant for each element of $\overline{W}_n$, and this will only be the case if at least one eigenvalue is equal to 1 and no eigenvalues are outside the unit circle. A steady-state model that does not have such an eigenvalue spectrum should be viewed with skepticism. However, the truncation errors that occur in numerical calculations of the eigenvalues may perturb the eigenvalue at 1 and cause the solution that is calculated on the basis of these approximate eigenvalues to be either stable or unstable.

When A has eigenvalues that are neither simple nor semisimple, then there is no similarity transformation between A and a diagonal matrix. However, there exists a similarity transformation between A and a matrix in Jordan canonical form

$$A = K J K^{-1}$$

where K is now a matrix of generalized eigenvectors. One obtains as above

$$Q_{n+1} = J Q_n$$

but each Jordan block contains a set of coupled first-order difference equations of the form

$$q_{n+1} = \begin{pmatrix} \lambda & 1 & 0 & \cdots & 0 & & \\ 0 & \lambda & 1 & \cdots & 0 & & \\ \vdots & & \vdots & \vdots & \ddots & \vdots & \\ 0 & 0 & 0 & \cdots & \lambda & & \end{pmatrix} q_n$$

The equation for the last element $q_{M,n}$ is solved to give $q_{n,M} = \lambda^n q_{0,M}$. The equation for the second-to-last element now becomes

$$q_{n+1,\,M-1} = \lambda q_{n,\,M-1} + q_{0,\,M}\lambda^n$$

which is a linear, first-order, inhomogeneous, finite-difference equation. The solution is found by standard techniques to be $q_{n,M-1} = q_{0,M-1}\lambda^n + q_{0,M}\, n\lambda^{n-1}$, where the first term in the expression is the homogeneous solution and the second term is a particular solution. The third-to-last element satisfies

$$q_{n+1,\,M-2} = \lambda q_{n,\,M-2} + q_{0,\,M-1}\lambda^n + q_{0,\,M} n\lambda^{n-1}$$

which again is a linear, first-order, inhomogeneous, finite-difference equation. It is obvious how one now proceeds to find the solutions for all the $q_{n,m}$.

An important point to note about the analytical solution is that changing the initial condition by multiplying by a constant changes the solution by the same factor. This is, of course, as expected. The population balance is homogenous and, unless the normalization criteria or a substrate balance is used, the solution is determined only up to an arbitrary constant.

Another point worth noting about the analytical solution is that it shows that the transient distribution over a subinterval of size Δ is a linear combination of all the initial subinterval distributions. This greatly restricts the possible transient solutions and in particular makes it impossible for the system to reach the true steady-state distribution if the initial distributions are such that a linear combination of them cannot equal the steady-state distribution. This point as well as the analytical solution method are illustrated in the example below.

Example 4.9: Transients with two control points for division Consider an organism growing in a chemostat with dilution rate D, for which half the cells divide at age a_1 and the remaining cells all divide at age 2. The true steady-state solution, the solution to the steady-state population balance model, will be needed for comparison purposes. Its normalized version is

$$f_{ss}(a) = \begin{cases} 2De^{-Da}, & 0 < a < a_1 \\ De^{-Da}, & a_1 < a < 2 \end{cases} \tag{4.14}$$

where the linkage equation is

$$1 = e^{-Da_1} + e^{-2D}$$

We get the smallest possible number of subdivisions of the age axis if we pick $a_1 = 1$, in which case $\Delta = 1$, $\Phi_1 = 0.5$ and $\Phi_2 = 1$. The matrix A becomes

$$A = \begin{pmatrix} e^{-D} & 2e^{-D} \\ 0.5e^{-D} & 0 \end{pmatrix}$$

We want to pick the value of the dilution rate that gives us a steady state, so e^{-D} is the root of the linkage equation

$$1 = e^{-D} + (e^{-D})^2$$

Only the positive root of this equation gives a physically meaningful value for D, so

$$e^{-D} = \frac{\sqrt{5}-1}{2}$$

giving the equation

$$\overline{W}_{n+1} = \begin{pmatrix} \frac{\sqrt{5}-1}{2} & \sqrt{5}-1 \\ \frac{\sqrt{5}-1}{4} & 0 \end{pmatrix} \overline{W}_n \tag{4.15}$$

An eigenvalue analysis of the A matrix provides the similarity transformation below

$$\overline{W}_{n+1} = \begin{pmatrix} 1-\sqrt{5} & 1 \\ 1 & \frac{\sqrt{5}-1}{4} \end{pmatrix} \begin{pmatrix} \frac{\sqrt{5}-3}{2} & 0 \\ 0 & 1 \end{pmatrix} \begin{pmatrix} \frac{\sqrt{5}-1}{2(\sqrt{5}-5)} & \frac{2}{5-\sqrt{5}} \\ \frac{2}{5-\sqrt{5}} & \frac{2(\sqrt{5}-1)}{5-\sqrt{5}} \end{pmatrix} \overline{W}_n$$

or, equivalently,

$$Q_{n+1} = \begin{pmatrix} \frac{\sqrt{5}-3}{2} & 0 \\ 0 & 1 \end{pmatrix} Q_n \Rightarrow Q_n = \begin{pmatrix} \left(\frac{\sqrt{5}-3}{2}\right)^n & q_{0,1} \\ & q_{0,2} \end{pmatrix}$$

Backtransforming the solution gives

$$\overline{W}_n = \begin{pmatrix} 1-\sqrt{5} & 1 \\ 1 & \frac{\sqrt{5}-1}{4} \end{pmatrix} \begin{pmatrix} \left(\frac{\sqrt{5}-3}{2}\right)^n & q_{0,1} \\ & q_{0,2} \end{pmatrix} =$$

$$\begin{pmatrix} (1-\sqrt{5})\left(\frac{\sqrt{5}-3}{2}\right)^n q_{0,1} + q_{0,1} \\ \left(\frac{\sqrt{5}-3}{2}\right)^n q_{0,1} + \left(\frac{\sqrt{5}-1}{4}\right) q_{0,2} \end{pmatrix}$$

The two constants $q_{0,1}$ and $q_{0,2}$ must be determined from the initial condition. In order to obtain any interesting dynamics, this initial condition must evidently be different from the steady-state distribution. We will use a constant value of $f(a, 0) = ½$. Therefore $q_{0,1}$ and $q_{0,2}$ satisfy the equation

$$\begin{pmatrix} \frac{1}{2} \\ \frac{1}{2} \end{pmatrix} = \begin{pmatrix} (1-\sqrt{5})q_{0,1} + q_{0,2} \\ q_{0,1} + \left(\frac{\sqrt{5}-1}{4}\right)q_{0,2} \end{pmatrix} \Rightarrow \begin{pmatrix} q_{0,1} \\ q_{0,2} \end{pmatrix} = \begin{pmatrix} \frac{1}{4} \\ \frac{\sqrt{5}+1}{4} \end{pmatrix}$$

So, finally

$$\overline{W}_n = \begin{pmatrix} \frac{1+\sqrt{5}}{4} + \left(\frac{\sqrt{5}-3}{2}\right)^n \frac{1-\sqrt{5}}{4} \\ \frac{1}{4}\left(1 + \left(\frac{\sqrt{5}-3}{2}\right)^n\right) \end{pmatrix} \tag{4.16}$$

The steady state predicted by this solution is obtained in the limit as n goes to infinity. Clearly

$$\overline{W}_\infty = \begin{pmatrix} \frac{1+\sqrt{5}}{4} \\ \frac{1}{4} \end{pmatrix} \tag{4.17}$$

which, after normalization, gives

$$f(a) = \begin{cases} 3-\sqrt{5}, & 0 < a < 1 \\ \sqrt{5}-2, & 1 < a < 2 \end{cases}$$

which, obviously, is different from the true steady-state solution, Eq. (4.14).

The result is plotted for $n = 1$, $n = 2$, and $n = \infty$ together with the true steady-state solution, Eq. (4.14), in Fig. 4.18. Although the transient solution rapidly approaches its steady-state solution, this solution is very different from the true steady-state solution. The reason for the poor final result is, of course, that the initial condition is a constant. The transient solution, being a linear combination of the initial distributions, must therefore be constant over each subinterval and the best one can hope for is a steady-state solution that approximates the true exponential solution by a step function with only two steps. Considering this constraint, the steady-state solution is quite reasonable.

Furthermore, one must not confuse the true steady-state solution, Eq. (4.14), with the steady-state solution in Eq. (4.17). The former is the solution to the steady-state PBM, while the latter is the steady-state solution to a finite-difference equation, Eq. (4.15). This finite-difference equation models only the distribution of states at times equal to integer multiples of the step size Δ, and, even at steady state, the solution between these times may not equal the steady-state solution to the difference equation.

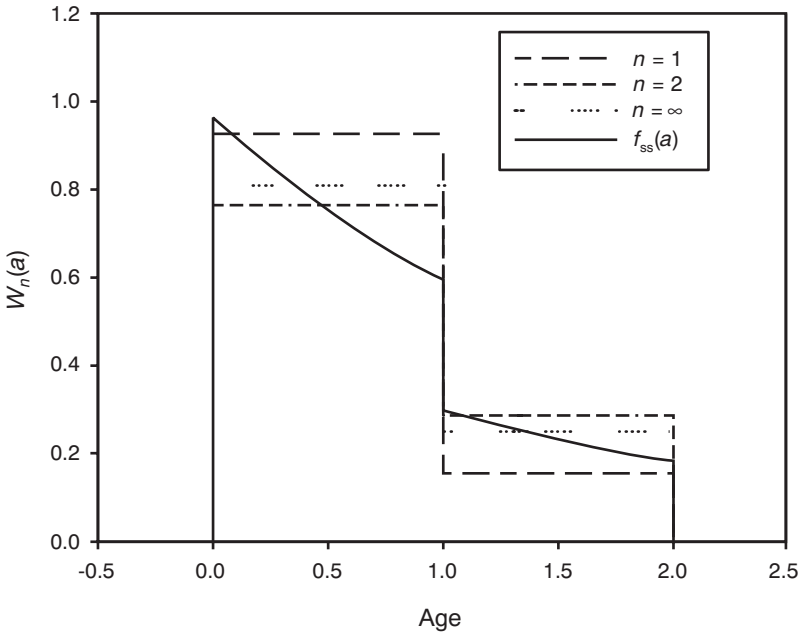

Figure 4.18 The solution in Eq. (4.16) for $n = 1$, $n = 2$, and $n = \infty$, together with the true steady-state solution, $f_{ss}(a)$.

For instance, using the steady-state solution to the finite-difference equation, Eq. (4.17), as initial condition for the age population balance, one finds the transient solution between $t = 0$ and $t = \Delta = 1$ to be

$$W(a, t) = \begin{cases} \dfrac{3+\sqrt{5}}{4} e^{-Dt}, & 0 < a < t \\ \dfrac{1+\sqrt{5}}{4} e^{-Dt}, & t < a < 1 \\ \dfrac{1+\sqrt{5}}{8} e^{-Dt}, & 1 < a < 1 + t \\ \dfrac{1}{4} e^{-Dt}, & 1 + t < a < 2 \end{cases}$$

where the physical age a has been reintroduced instead of the translated age $\hat{a}$. This solution is best appreciated by viewing it as an animation as time increases from 0 to 1. Readers who can create this animation easily on available software are strongly encouraged to do so. In a book, plots of the solution at various times, Fig. 4.19, will have to suffice.

The initial solution is a step function consisting of two steps. These two steps move toward higher ages as time increases, while simultaneously decreasing in magnitude as a result of washout of cells from the reactor. At the same time, cell divisions at the two control points at $a = 1$ and $a = 2$ maintain

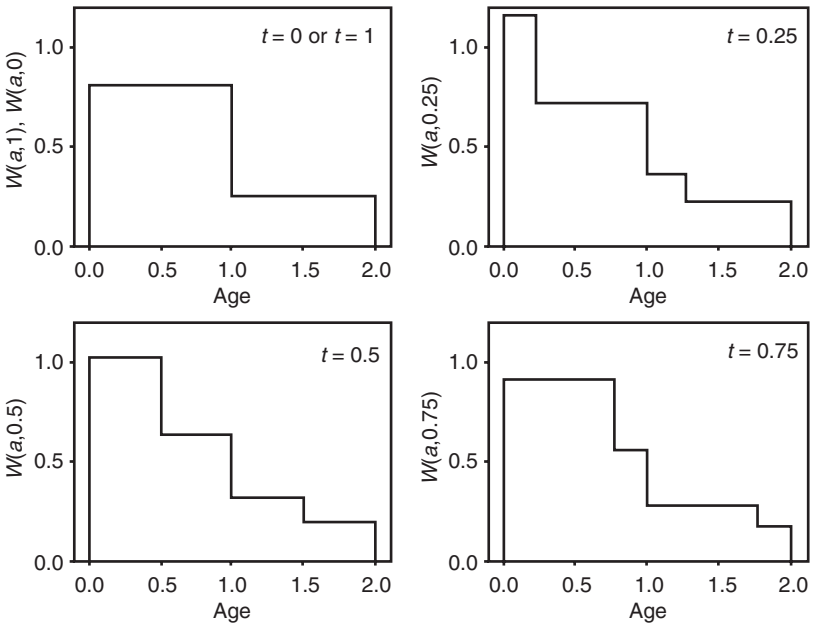

Figure 4.19 Transient solution obtained when the steady-state solution in Eq. (4.17) is used as the initial condition to the PBM. The solution repeats with a period of $\Delta = 1$.

the discontinuous steps at these points. The overall effect is that of two step functions, one stationary and one continuously moving toward higher ages. At times equal to integer multiples of Δ, the discontinuous points on the two step functions coincide perfectly, creating a distribution with only two steps. Thus, the transient solution is periodic with the period $\Delta = 1$.

It may seem strange that the transient solution to the PBM does not approach the solution to the steady-state PBM as time becomes large, but instead approaches a periodic function. One might at first suspect that the steady state is unstable and the solution approaches a limit cycle. However, this is not the case. As we shall see forthwith, the solution that was found above is not a limit cycle because it is altered when the initial condition is changed and is therefore not the general limit of the system at large times. The dynamic situation can be compared to that of a frictionless pendulum. Once started, the pendulum will never stop swinging, but the magnitude of the back and forth movement will depend on how the swinging of the pendulum was started. The pendulum, being frictionless, will also never reach the steady state and hang straight down without moving. This is so because, without friction, there is no way to dissipate kinetic energy once it is imparted to the pendulum. Similarly, the population balance model with divisions only at ages 1 and 2 has transient solutions that are linear combinations of the initial distributions over each of the subintervals $0 < a < 1$ and $1 < a < 2$. The model does not have a mechanism for altering the shape of these distributions

and therefore has no mechanism for reaching the solution of the steady-state population balance. This is a problem that will occur in all control point models. They are inherently incapable of exhibiting a dynamics that is sufficiently rich to allow a system to reach the true steady state, except in very special cases, and will therefore tend toward an oscillatory solution as time becomes large.

The fact that the transient distributions are linear combinations of the initial distributions over the subintervals that arise in the solution procedure described above suggests that a better model of the transient dynamics can be obtained by picking a more reasonable initial condition. We do know that the steady-state solution is an exponential function, so to get an initial condition that can represent this fact, pick the normalized function

$$f(a, 0) = \frac{D}{1 - e^{-2D}} e^{-Da} \qquad 0 < a < 2$$

Partitioning this function into the two subintervals and translating the age variable of the second interval to an age between 0 and 1 gives the initial condition

$$\begin{pmatrix} W_1(\hat{a}, 0) \\ W_2(\hat{a}, 0) \end{pmatrix} = \begin{pmatrix} \dfrac{D}{1 - e^{-2D}} e^{-D\hat{a}} \\ \dfrac{De^{-D}}{1 - e^{-2D}} e^{-D\hat{a}} \end{pmatrix}$$

and the two constant $q_{0,1}$ and $q_{0,2}$ therefore satisfy

$$\begin{pmatrix} \dfrac{D}{1 - e^{-2D}} e^{-D\hat{a}} \\ \dfrac{De^{-D}}{1 - e^{-2D}} e^{-D\hat{a}} \end{pmatrix} = \begin{pmatrix} (1 - \sqrt{5}) q_{0,1} + q_{0,2} \\ q_{0,1} + \left(\dfrac{\sqrt{5} - 1}{4}\right) q_{0,2} \end{pmatrix}$$

giving, after considerable simplification steps,

$$\begin{pmatrix} q_{0,1} \\ q_{0,2} \end{pmatrix} = \begin{pmatrix} \dfrac{1}{4} + \dfrac{\sqrt{5}}{20} \\ \dfrac{7}{10}\sqrt{5} + \dfrac{1}{2} \end{pmatrix} De^{-D\hat{a}}$$

and

$$\overline{W}_n = \begin{pmatrix} (1 - \sqrt{5})\left(\dfrac{\sqrt{5} - 3}{2}\right)^n \left(\dfrac{1}{4} + \dfrac{\sqrt{5}}{20}\right) + \dfrac{7}{10}\sqrt{5} + \dfrac{1}{2} \\ \left(\dfrac{\sqrt{5} - 3}{2}\right)^n \left(\dfrac{1}{4} + \dfrac{\sqrt{5}}{20}\right) + \dfrac{15 - \sqrt{5}}{20} \end{pmatrix} De^{-D\hat{a}} \tag{4.18}$$

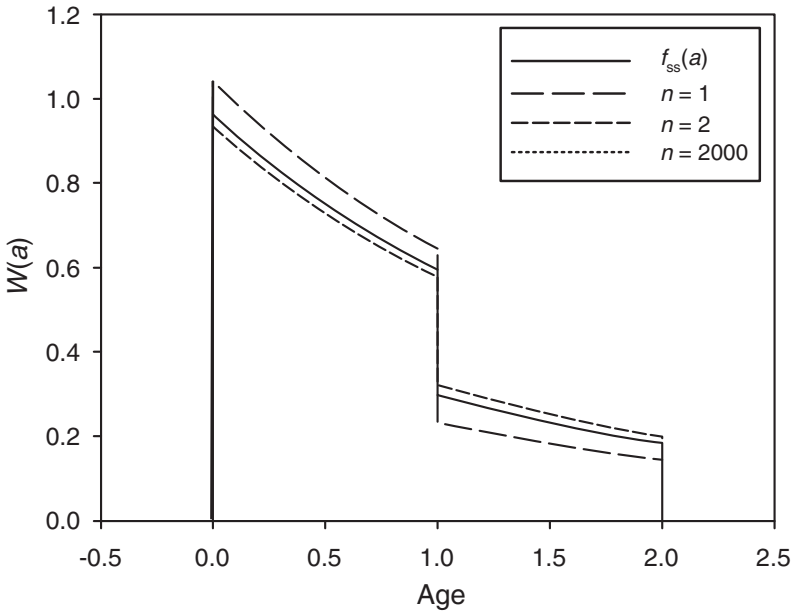

Figure 4.20 The solution in Eq. (4.18) for $n = 1$, $n = 2$, and $n = 2000$, together with the steady-state solution, $f_{ss}(a)$. The last two graphs are, for practical purposes, identical.

The solution at large times approaches

$$\overline{W}_{\infty} = \begin{pmatrix} \frac{7}{10}\sqrt{5} + \frac{1}{2} \\ \frac{15 - \sqrt{5}}{20} \end{pmatrix} De^{-D\hat{a}}$$

which, after normalization, does give the same solution as the true steady-state solution, Eq. (4.14). Readers are urged to do the calculations necessary to convince themselves of this. Solutions for several values of n are plotted in Fig. 4.20.

Using an initial condition with the same functional forms as the steady-state solution gives a transient that is more intuitively convincing than the transients that follow from other initial conditions because this transient does approach the true steady-state distribution. However, the transient shape of the distribution is still quite limited because it is, at all times, a linear combination of just two initial distributions. One can overcome this limitation by a minor change in the problem, a change that results in a smaller size of the interval Δ.

For instance, one can pick $a_1 = 1.02$. Although only a small change in the value of this model parameter, this change in a_1 forces a change in Δ from 1 to 0.02, resulting in a solution that is in many ways more intuitively appealing. The new, smaller value of Δ results in 100 subintervals, as opposed to

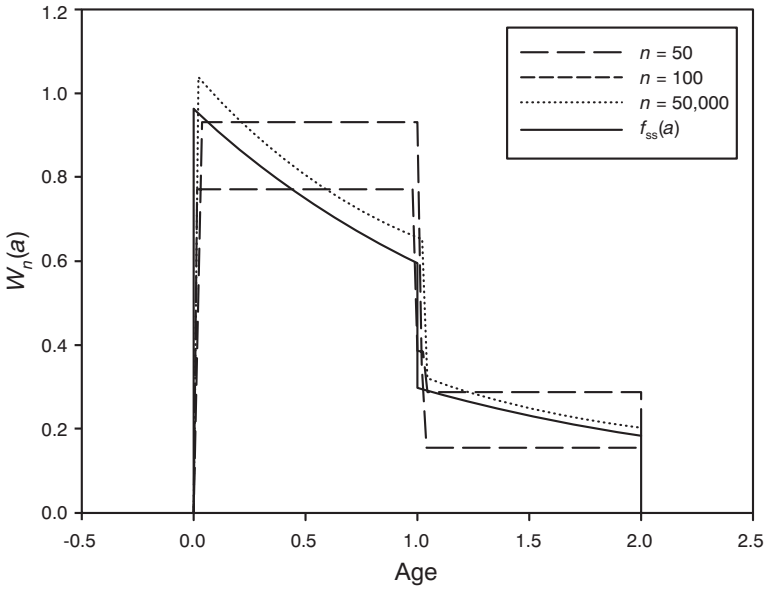

Figure 4.21 The solution for the case $a_1 = 1.02$ for $n = 50$, $n = 100$, and $n = 50{,}000$, together with the steady-state solution $f_{ss}(a)$.

two in the model considered above, and the transient over each subinterval is therefore a linear combination of 100 initial distributions, opening up the possibility of much richer dynamics. To solve Eq. (4.12) one must now find the eigenvalues and eigenvectors of a 100-by-100 matrix. The only Φs that are not equal to zero are $\Phi_{51} = 0.5$ and $\Phi_{100} = 1$, so the matrix is quite sparse, yet it is not feasible to find the eigenvalues and eigenvectors symbolically. However, there are well-known and powerful methods for doing this numerically, and they are available on most mathematical platforms. Such numerical results, for the constant initial condition $f(a, 0) = ½$, are plotted in Fig. 4.21 The values of n are chosen to show the distribution at the same dimensionless times as in Fig. 4.18

In this case, the distributions at $n = 50$ and $n = 100$ are qualitatively very similar to the distributions at $n = 1$ and $n = 2$ for the model with $a_1 = 1$, and, as for this model, the distribution at large times is a piecewise constant function. However, the smaller value of Δ and the concomitant larger number of intervals provides for a much better approximation to $f_{ss}(a)$ at large times. However, the transient still does not reach the true steady state, as it can not possibly do, given the constant initial condition, but oscillates with the period Δ.

The two models, the model with $a_1 = 1$ and the model with $a_1 = 1.02$, both with the constant initial condition, give different transients and very different periodic solutions at large times. This is disturbing because one would expect that a small change in a model parameter value would give a small change in the model solution. Yet, in this case, the opposite holds. An even

smaller change, say setting $a_1 = 1.002$, will result only in a transient that is even more different from the $a_1 = 1$ transient than the $a_1 = 1.02$ transient. The solution at large times will oscillate with an even smaller period of $\Delta = 0.002$ and will be very close to the true steady-state solution at all times. In fact, as Δ becomes smaller and the number of subintervals required by the solution method becomes larger, the ability of the solution to approximate the true steady-state solution becomes better. Thus, the model solution does not depend in a smooth manner on the value of the model parameter a_1. An arbitrary small change from $a_1 = 1$ will have a huge effect on the solution. Of course, this happens because the solution domain tiling is not a smooth function of a_1 and each solution is valid for only one specific tiling and value of a_1. In other words, each a_1 value is a bifurcation point for the transient solution.

These solution properties—the inability to eliminate oscillations in the transient, except for very specific initial conditions, and the fact that a specific oscillatory solution exists only at an isolated point in the parameter space—are absurd from a biological point of view. The absurd properties occur because the control point model does not fully portray what happens in the population.

In actual cell populations, divisions occur at random over a continuous interval of ages or cell states. The descendants of two cells that are initially in very similar or even identical states can therefore have very different cell states. This process may be thought of as a gradual mixing state space so that descendants of any cell will have states that are distributed over the entire range of cell states.

In the control point model, on the other hand, cells that initially have cell states such that they are located in the same subinterval, as these intervals appear in the solution procedure, will remain a constant distance from one another in state space. Thus there is no mixing of state space within any of the subintervals. It is this absence of a complete mixing of state space that causes control point models to exhibit these biologically absurd solutions.

As demonstrated in this example, the absurd solution properties can be alleviated to some extent by choosing model parameters that give a fine, rather than a coarse, tiling of solution domains. The control point model can be regarded as an approximation to the biologically more reasonable model with a continuous distribution of division states, and the finer the domain tiling is, the better this approximation is.

The example above, division at only two control points, is rather contrived but serves to illustrate the method. In the next example, we will calculate the transient age distribution for a more realistic case, but first we must address the issue of how one might reasonably approximate an arbitrary division intensity by a sequence of division control points.

A reasonable way is to first approximate the division intensity by a staircase function. For instance, pick a sequence of ages $\{a_n\}_{n = 1 \cdots N}$

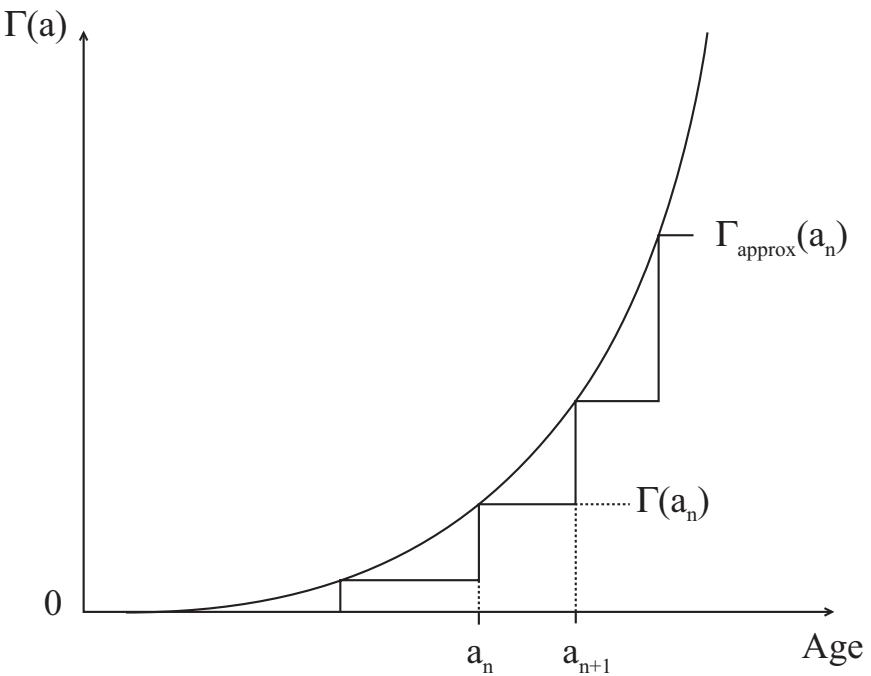

Figure 4.22 Approximation of the division intensity by a staircase function.

that will become the ages at the control points and let the staircase approximation of the division intensity be, as in Fig. 4.22,

$$\Gamma_{\text{approx}} = \Gamma(a_n), \quad a_n < a < a_n + 1$$

With the approximate division intensity, the age distribution between a_n and a_{n+1}, at steady state in a chemostat is

$$W(a) = W(a_n) e^{-\Gamma(a_n)(a - a_n)} e^{-D(a - a_n)}$$

The two exponential terms indicate the fraction of cells that did not divide between a_n and a and the fraction of cells that did not wash out of the reactor. We will let Φ_n, the fraction of cells that divide at the control point at a_n, equal the fraction of cells that divide between a_n and a_{n+1} when the staircase approximation of the division intensity is used. Thus

$$\Phi_n = 1 - e^{-\Gamma(a_n)(a - a_n)} \tag{4.19}$$

Example 4.10: Decay of synchrony Assume a division intensity of the form

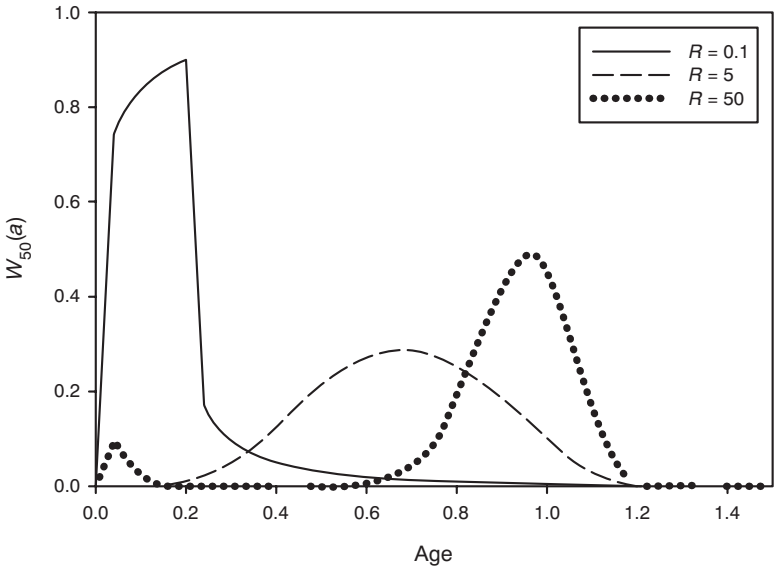

Figure 4.23 The age distribution at dimensionless time 2 for $R = 0.1$, $R = 5$, and $R = 50$.

$$\Gamma(a) = \begin{cases} 0, & a < 1 \\ R\dfrac{a-1}{2-a}, & 1 < a < 2 \end{cases}$$

and approximate this by using Eq. (4.19) and equidistant control points between 1 and 2.

To set up the difference equation for the transient, first solve for the dilution rate that gives a steady state using the linkage equation, then set up the matrix A and find the eigenvectros and eigenvalues, all of which is just tedious protocol. Results for various choices of model parameter values are shown in the following figures, using as initial condition a pulse of synchronized cells between ages 0 and 0.2,

$$W_0(a) = \begin{cases} 5, & 0 < a < 0.2 \\ 0, & 0.2 < a \end{cases}$$

Twenty-five equidistant control points between 1 and 2 were used in all cases. Fig 4.23 shows the distribution at a dimensionless time of 2 or $n = 50$, the earliest time after which all cells initially present must have divided once. The distribution is shown for $R = 0.1$, $R = 5$, and $R = 50$.

Notice how the synchrony is better maintained for the high and low values of R. At these two extremes, the bulk of the cells divide at ages either close to 2 or close to 1, resulting in minimal loss of synchrony. This is also reflected in the position of the two distributions at this time. The distribution for

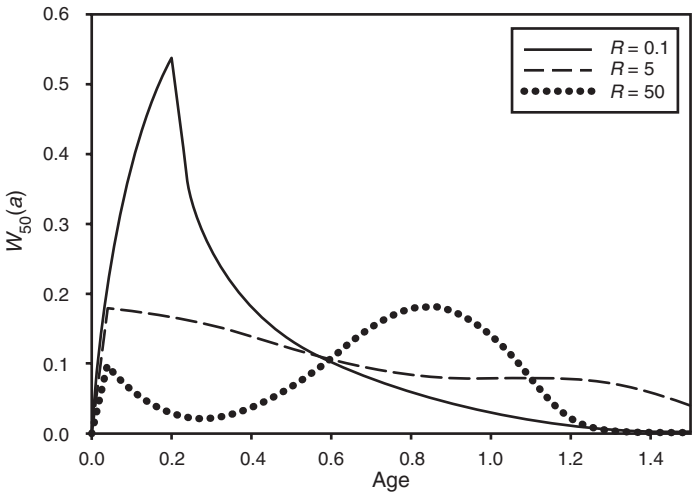

Figure 4.24 The age distribution at dimensionless time 10 for $R = 0.1$, $R = 5$, and $R = 50$.

$R = 0.1$ is located at young ages, reflecting the fact that these cells just divided, i.e., at ages close to 2, while the distribution for $R = 50$ is centered around the age 1, reflecting the fact that these cells divided around an age of 1. At intermediate values of R the divisions are spread more evenly over the interval from 1 to 2, causing a greater loss of synchrony.

The same observations can be made at a dimensionless time of 10 Fig. 4.24 The case for which $R = 5$ has almost attained the steady-state distribution, while the two other cases still have a significant amount of synchrony.

4.6 Problems

4.1. Solve

$$-x_1\frac{\partial \omega}{\partial x_1} + (x_2 + 1)\frac{\partial \omega}{\partial x_2} = -x_1\,\omega + x_2$$

where the initial condition is given along the x_1 axis as $w_0(x_1)$. The final result does contain a difficult integral, which you do not have to evaluate.

4.2. Solve

$$-x_1\frac{\partial \omega}{\partial x_1} + (x_2 + 1)\frac{\partial \omega}{\partial x_2} + \frac{\partial \omega}{\partial x_3} + 2x_3\frac{\partial \omega}{\partial x_4} = -x_3\,\omega + x_1$$

with the boundary condition that w is given as some function w_0 in (x_1, x_3, x_4) space for $x_2 = 0$. That is,

$$\omega(x_1, 0, x_3, x_4) = \omega_0(x_1, x_3, x_4)$$

The final result does contain a difficult integral, which you do not have to evaluate.

4.3. Solve the wave equation

$$\frac{\partial^2 \omega}{\partial t^2} - \gamma^2 \frac{\partial^2 \omega}{\partial x^2} = 0$$

for a wave reflection at $x = 0$ using d'Alembert's method. For simplicity consider only waves on the positive x axis and assume the initial conditions below are valid.

$$\omega(x, 0) = \omega_0(x), \qquad \left(\frac{\partial \omega}{\partial t}\right)_{x,\,0} = 0, \qquad \omega(0, t) = 0$$

where clearly one must demand that $w_0(0) = 0$ and $w_0(x)$ are defined only for positive values of x.

This problem is not as straightforward as it might seem. The initial manifold is the positive x axis, but the solution obtained from this manifold is not valid for all positive arguments of x and t because w_0, which appears in the solution, is defined only for positive arguments. Figuring out how to solve this puzzle is the main motive for this assignment.

4.4. Solve

$$\frac{\partial W}{\partial t} + \frac{\partial \nu\, mW}{\partial m} = -(D + \Theta(m))W(t, m), \qquad W(0, m) = W_0(m)$$

This model can be interpreted as the cell mass population balance equation of a population of cells that have lost the ability to divide, but still grow, in this case by first-order single-cell kinetics, and die according to some death intensity Θ.

4.5. For a binary fission organism that divides at the cell mass $2M$ and with a single-cell mass growth rate that follows zeroth-order kinetics with the rate constant k, the steady-state mass distribution in a chemostat is

$$f(m) = \frac{\ln(16)}{M} e^{-\ln(2)m/M}, \quad M < m < 2M$$

and

$$D = \frac{k}{M} \ln(2)$$

Suppose now that the dilution rate in the chemostat is increased to

$$D = 0.75\frac{k}{M}$$

but that the organism is already growing at its maximum rate, i.e., that k and M do not change. Follow the washout and the effect on the shape of the distribution of states by calculating $W(m, t)$ for the first couple of generations after the increase in the dilution rate, assuming an arbitrary initial distribution $W_0(m)$.

Examine the solutions obtained for the first couple of domains and come up with an educated guess for the solution in the nth domain. Prove by induction that the guessed solution is, in fact, the correct solution (assuming of course that you have guessed correctly).

4.6. Solve for the transient age distribution for times up to $2a_2$ for a dilution rate shift up if the age at division changes according to

$$a_d = \begin{cases} a_1 - (a_1 - a_2)\dfrac{t}{2a_2}, & t < 2a_2 \\ a_2, & t > 2a_2 \end{cases}$$

and the initial condition is the steady-state age distribution in a population with division age a_1. Find the solution up to a time equal to $2a_2$. Plot the dimensionless age distribution when $a_2 = a_1/2$ and when $a_2 = a_1/10$. Make diagrams, to scale, of the solution domains for these two cases and, on the basis of these diagrams, give a qualitative explanantion for the shape of the age distributions at $t = 2a_2$.

4.7. Consider the transient age distribution in a dilution rate shift down in which the age at division changes according to

$$a_d = \begin{cases} a_1 + (a_2 - a_1)\dfrac{t}{\tau}, & t < \tau \\ a_2, & t > \tau \end{cases}$$

Solve the following specific cases:

A. $\tau = a_2$ and find the solution up to $t = \tau$,

B. $\tau = 2a_2$ and find the solution up to $t = \tau$.

In all cases, use the steady-state age distribution in a culture with division age a_1 as the initial condition. Draw the solution domains and discuss, on the basis of the drawings, the qualitative transient for the cases when $\tau = a_2/2$ and $a_1 = a_2/2$, $a_2/4$, and $3a_2/4$.

4.8. Consider a situation in which the age at division changes as follows: Between times 0 and τ, it increases linearly from $a_1 = 1.8\,\tau$ to $a_2 = 2.2\,\tau$.

Between times τ and 2τ, it decreases linearly from a_2 to a_1. Let the age distribution at time 0 be $W_0(a)$ and find the transient age distribution up to time 2τ in a batch culture. From your result, deduce an equation for the age distribution after n periods, $W_n(a)$, in terms of the age distribution after $n-1$ periods, $W_{n-1}(a)$, assuming that the age at division changes as described above. Write a program that calculates and plots $W_n(a)$ for any value of n, using this recursive formula. To illustrate the transient dynamics, you may also want to plot a surface plot or contour plot of $W_n(a)$ as a function of a and n. Any such plot will be dominated by the part that represents the age distribution at the highest value of n unless the distributions are scaled in some way. Instead of normalizing the distribution for each value of n, it is simpler to multiply the values of $W_n(a)$ by 2^{-n}. It will hopefully become clear to the reader why this scaling policy works well.

4.9. Consider an organism for which half the cells divide at age 1 and the remaining cells divide at age 2. Let the initial age distribution be $W_{0,1}(a)$ for $a < 1$ and $W_{0,2}(a)$ for $a > 1$ and find the age distribution for times up to 3. This appears to be a very tedious problem, and to some extent it is. However, the point of doing this calculation is to see that, after finding the solution in the first couple of domains, the calculations get to a point where they become so repetitive that one can fairly easily extend the calculations to higher times by "copying" previous results. In fact, if you do this, finding the solution to time 4 or 5 involves only slightly more work than finding the solution up to time 3.

4.10. Assume that cells in a population first start to divide when they reach the age A and that all cells have divided by the age $2A$. The simplest expression for the division intensity $\Gamma(a)$ that satisfies this behavior is an expression of the form

$$\Gamma(a) = \begin{cases} 0, & a < A \\ R\dfrac{a-A}{2A-a}, & a > A \end{cases}$$

where R is a positive model parameter.

Use this model to explore the gradual loss of synchrony through consecutive cell divisions. I suggest you proceed as follows: Start by simplifying the problem a bit by making time and cell age dimensionless by division with A. As initial condition, assume a narrow, normalized rectangular distribution of cells between ages 0 and ε; i.e., assume that

$$W(t=0,\, a) = W_0(a) = \begin{cases} \dfrac{1}{\varepsilon}, & a < \varepsilon \\ 0, & a > \varepsilon \end{cases}$$

Then solve for the age distribution at dimensionless times 2, 4, etc. (as long as you can stand it), and compare these distributions by plotting them together. Plot the cell number, the zeroth moment of the age distribution, versus time. Discuss the possibility of obtaining $\Gamma(a)$ from measurements of the cell number versus time in cultures that are initially synchronized.

After a sufficiently long time, the shape of the age distribution will not change. One can then write

$$W(t, a) = Ce^{\mu t} f(a)$$

where μ is the specific growth rate of the population and $f(a)$ is the normalized age distribution. Find μ and $f(a)$ and plot $f(a)$ together with the normalized distributions found in the previous question.

The next two problems have nothing whatsoever to do with population balances. They are examples of pathological problems where blind, uncritical use of Cauchy's method gives problems. Working through the solution may be a help in coming to a firmer understanding of the solution structure of first-order linear PDEs.

4.11. Discuss solution of

$$\frac{\partial W}{\partial t} - (t + x)\frac{\partial W}{\partial x} = 0$$

subject to the four different initial conditions below

$$W(0, x) = W_0(x), \quad x \in R$$

$$W(t, 0) = W_0(t), \quad t \in R$$

$$W(0, x) = W_0(x), \quad x \geq 0 \quad \text{and} \quad W(t, 0) = t + 1, \quad t \geq 0$$

$$W(0, x) = x, \quad x \geq 0 \quad \text{and} \quad W(t, 0) = 1 + e^t(t - 1), \quad t \geq 0$$

4.12. Discuss the solution of

$$\frac{\partial W}{\partial t} + 2\sqrt{x}\frac{\partial W}{\partial x} = F(t, x)$$

subject to

$$W(0, t) = W_0(t), \quad t \in R$$

for $F(t, x) = 0$, $F(t, x) = 1$, and $F(t, x) = t$.

Chapter

5

Cell Cycle Synchrony

A growing cell culture is considered synchronous if cell divisions occur approximately simultaneously for all cells—in other words, if the specific rate of cell division is not constant but alternates between low values, corresponding to an absence of cell divisions, and high values, corresponding to the synchronous cell divisions. Thus, in principle at least, any batch culture that is not in steady exponential growth or any continuous culture that is not in steady-state growth is partially synchronized.

The better the cell divisions are synchronized, the higher the "degree" or percent of synchrony is said to be. Although many attempts have been made to devise rigorous, quantitative measures of synchrony [12, 29, 30, 83, 104], the terms *degree of synchrony*, *percent synchrony*, and *synchrony index* are often used as self-explanatory, qualitative terms without any attempt to define them rigorously. Obviously, cell cycle synchrony reflects a deviation of the distribution of states from the shape it would have under steady growth conditions and a single number, such as degree of synchrony, is generally insufficient to fully characterize such a deviation. Furthermore, unless steps are taken to maintain synchrony, cell cycle synchrony is typically lost within a few generations and the degree of synchrony therefore decreases continuously.

Synchronous cultures are primarily used as a tool for study of the cell cycle and, at the current rate of scientific progress in cell biology, can only be considered ancient, with books devoted to the subject appearing as early as the 1960s [15, 59, 105]. There are essentially two different methods for obtaining synchronous cultures: selection and induction synchrony. Selection synchrony, as the name implies, uses tools such as filtration, elution of bound cells, or sedimentation to isolate a

subpopulation of cells that are approximately all in the same state. The yield of synchronous cells by selection methods is typically low compared to the cell yield obtained by induction synchrony, the main topic of this chapter.

5.1 Induction Synchrony

Methods of induction synchrony force an entire population of cells into approximately similar cell states, using periodic environmental shifts, such as changes in temperature, nutrients, or illumination. Cell cycle blockers have also been used to arrest all cells in the same part of the cell cycle and a synchronous culture is obtained after removal of the block. Although induction synchrony has been used in numerous cell cycle studies, it has remained controversial, as it is argued that the division synchrony does not necessarily reflect synchrony of other aspects of the cell cycle and that the forcing required to achieve synchrony is so draconian as to render the synchronized cells unrepresentative of normal cells. For a recent contribution to this debate, see Refs. 23, 24, 88, and 89.

From a population balance perspective, induction synchrony can be explained as the outcome of periodic forcing of the population balance equation [39]. To develop a model of induction synchrony and to help understand the relevance of the timing and magnitude of the environmental shifts, consider a batch culture in which the age at division is forced, through some environmental means, to change with a period equal to 2 in the following manner:

$$a_d(t) = \begin{cases} 1.8 + 0.4t, & 0 < t < 1 \\ 2.2 - 0.4(t-1), & 1 < t < 2 \\ a_d(t-2), & t > 2 \end{cases} \tag{5.1}$$

We will find the solution for the first period of forcing. The solution domains are shown in Fig. 5.1 and the domain solutions are

Domain I: $$W_{\mathrm{I}}(a, t) = W_0(a - t), \quad t < a < a_d(t)$$

Domain II: $$W_{\mathrm{II}}(a, t) = 1.2\ W_0(1.8 - 0.6(t - a)), \quad t - 1 < a < t$$

Domain III: $$W_{\mathrm{III}}(a, t) = 2.8W_0(2.6 - 1.4(t - a)), \quad t - 13/7 < a < t - 1$$

Domain IV: $$W_{\mathrm{IV}}(a, t) = 3.36W_0(3.36 - 0.84(t - a)), \quad a < t - 13/7$$

from which the following recursion formula is obtained:

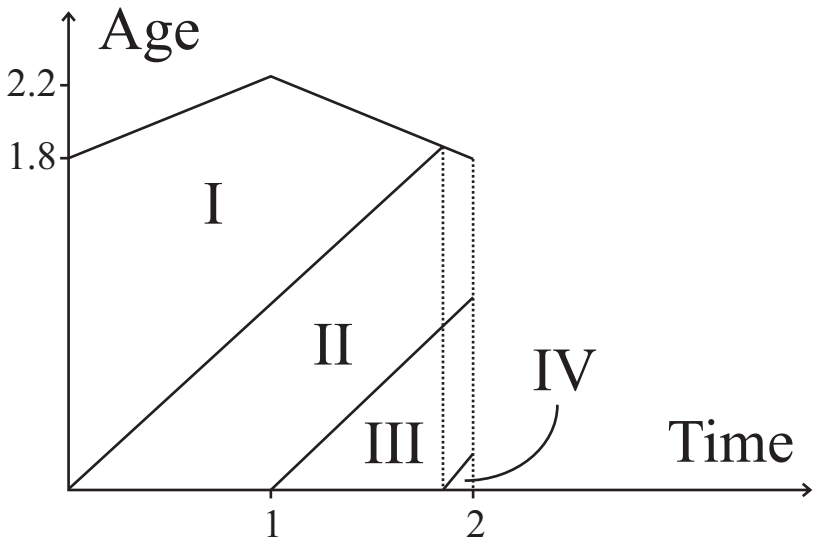

Figure 5.1 Solution domains for periodic forcing of division age.

$$W_{n+1}(a) = \begin{cases} 3.36\ W_n(1.68 + 0.84a), & a < 1/7 \\ 2.8\ W_n(1.4a - 0.2), & 1/7 < a < 1 \\ 1.2\ W_n(0.6 + 0.6a), & 1 < a < 1.8 \end{cases}$$

When the steady-state distribution for $a_d = 1.8$ is used as the initial condition for this recursion formula, the distributions in Fig. 5.2 are obtained. The onset of synchrony is evident.

The mechanism of induction synchrony can easily be understood in terms of the contractions and expansions of state space that occur over subsequent periods (Fig. 5.3).

When the magnitude and period of the forcing is such that the age at division equals the period of forcing at some point during the period, one can always identify a cell line that divides at an age equal to the period of forcing. In fact, one can find two such cell lines: one for which cell division occurs while the division age is a decreasing function of time, i.e., when state space is contracting, and another that divides while the division age is an increasing function of time, i.e., while the state space is expanding. These two cell lines are periodic in the sense that they represent cells that always divide at the same point in time during the period of forcing. All other growth curves experience a phase shift during each period. The phase shift is caused by the expansion and contraction of the state space and will therefore bring all nonperiodic

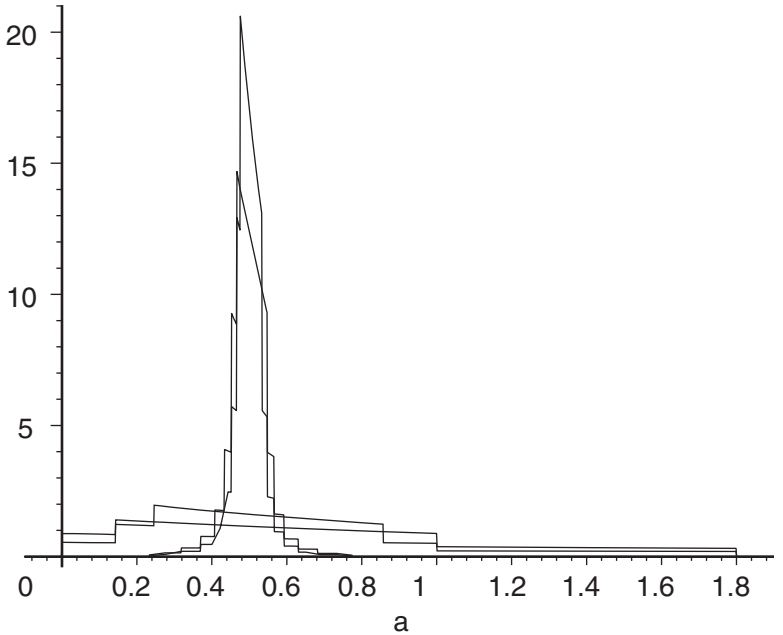

Figure 5.2 Age distributions after 1, 2, 8, and 9 periods of forcing, starting with the steady-state distribution. The distributions have been normalized by division by 2^n, where n is the number of periods since the start of periodic forcing.

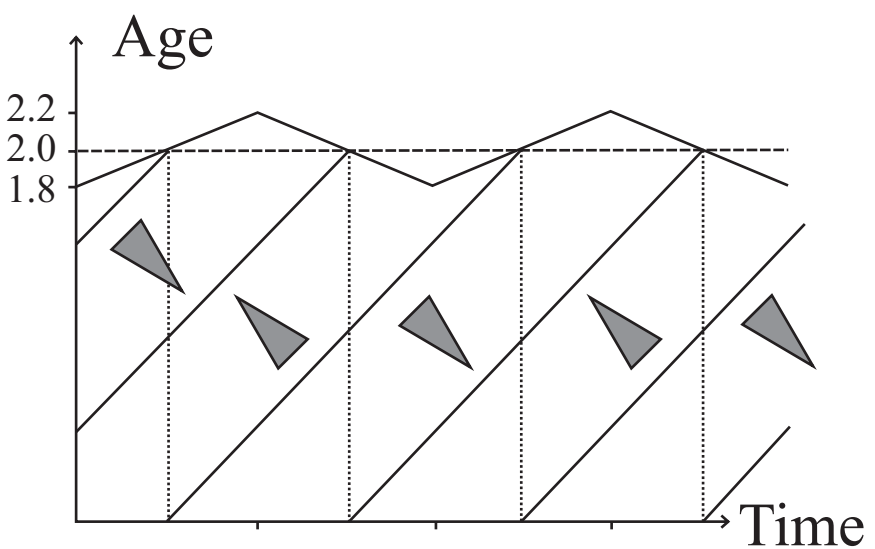

Figure 5.3 Attracting and repelling cell lines during periodic forcing. The attracting cell line is the curve that intersects the division age while the division age is decreasing. The repelling cell line is a curve that intersects the division age while the division age is increasing.

growth curves closer and closer to the periodic growth curve that divides while the division age is a decreasing function. This cell line is called *attractive* while the other periodic cell line is called *repelling*. Clearly, synchrony is induced only if an attractive cell line is formed during the periodic forcing. This in turn happens only when the division age equals the period of forcing at some point during the period. Thus, not all periodic environmental shifts can be expected to induce synchrony.

An entirely different way of looking at the phenomenon of induction synchrony is through derivation of iterated maps, functions that, in this case, relate the age of a cell before a period of forcing to the age after one period of forcing. Consider again the forcing function in Eq. (5.1). The cells present at time 0 can be partitioned into three groups, depending on how and when they divide during the following period of forcing (Fig. 5.4).

Cells in group I all divide while the division age is increasing. After this first division, the cells in group Ia undergo another division while the division age is decreasing, while the cells in Ib do not divide again. Cells in group II divide only once and all of them while the division age is decreasing.

Consider first the cells in group I. Let their age at time zero be a_n. The time of their first division t_1 is given by

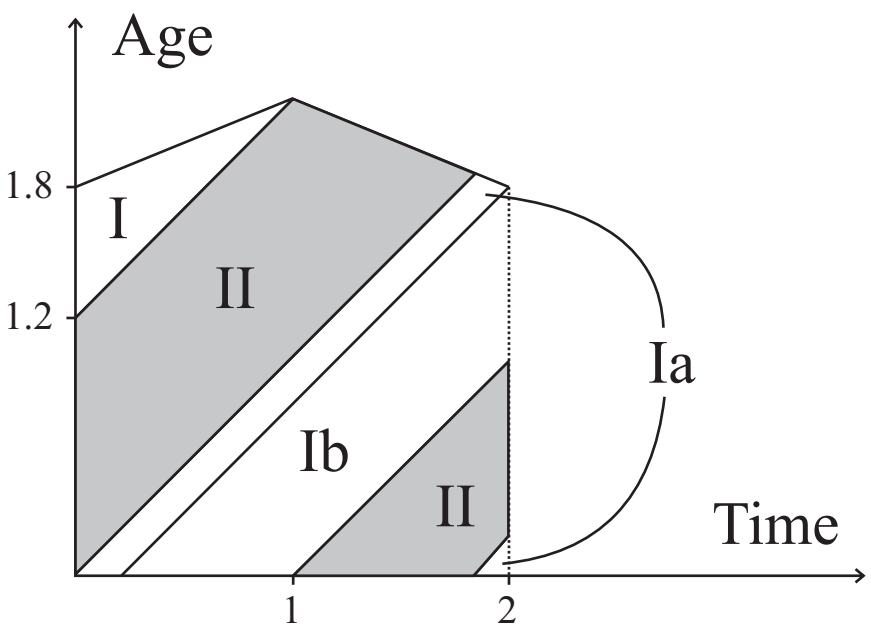

Figure 5.4 Partitioning of cells into groups depending on their division history. Cells in group I divide while the division age is increasing and split into two groups, Ia and Ib, depending on whether or not they undergo another division. Cells in group II divide only once and while the division age is decreasing.

$$a_d(t_1) = 1.8 + 0.4t_1 = a_n + t_1 \Rightarrow t_1 = 3 - {}^{5}\!/\!_{3}\, a_n$$

If $t_1 < 0.2$, these cells divide a second time, at t_2 given by

$$a_d(t_2) = 2.2 - 0.4\ (t_2 - 1) = t_2 - t_1 \Rightarrow t_2 = 4 - {}^{25}\!/\!_{21}\, a_n$$

The age at time 2 of the cells that divided twice is $a_{n+1} = 2 - t_2$, while the age of cells that divide only once is $2 - t_1$.

The cells in group II divide once, at t_1 given by

$$a_d(t_1) = 2.2 - 0.4\ (t_1 - 1) = a_n + t_1 \Rightarrow t_1 = {}^{13}\!/\!_{7} - {}^{5}\!/\!_{7}\, a_n$$

and their age at time 2 equals $2 - t_1$. Putting everything together, we obtain the following map between a cell's age before and after one period of forcing:

$$a_{n+1} = \begin{cases} {}^{1}\!/\!_{7} + {}^{5}\!/\!_{7}\, a_n, & 0 < a_n < 1.2 \\ {}^{5}\!/\!_{3}\, a_n - 1, & 1.2 < a_n < 1.68 \\ {}^{25}\!/\!_{21}\, a_n - 2, & 1.68 < a_n < 1.8 \end{cases}$$

This map is plotted in Fig. 5.5, where the broken diagonal line represents the identity map, $a_{n+1} = a_n$. The identity map intersects the map of cell ages at age values that do not change over a period, i.e., at the roots of the equation $a_{n+1}(a_n) = a_n$ or $a = 0.5$ and $a = 1.5$. This equation can be viewed as an instance of numerical root finding by direct substitution[1] and it is well known that initial guesses converge to the root if $\left| {}^{(da_{n+1}(x))}\!/\!_{dx} \right| < 1$. Rephrased for the situation at hand: Over several periods of forcing, all ages will converge to the root at $a = 0.5$ and diverge from the root at $a = 1.5$. Obviously, the root at $a = 0.5$ corresponds to the attracting cell line found above and the root at $a = 1.5$ to the repelling cell line.

5.2 Autonomous Oscillations

Autonomous oscillations have been observed in many microbial cultures, and in some cases, though certainly not all, these oscillations are associated with some degree of cell cycle synchrony. This phenomenon

[1]In direct substitution the roots of the equation $f(x) = 0$ are found by rewriting the equation in the form $x = g(x)$, providing an initial guess of the value of the root x_0 and improving this guess by iterations with $x_{n+1} = g(x_n)$.

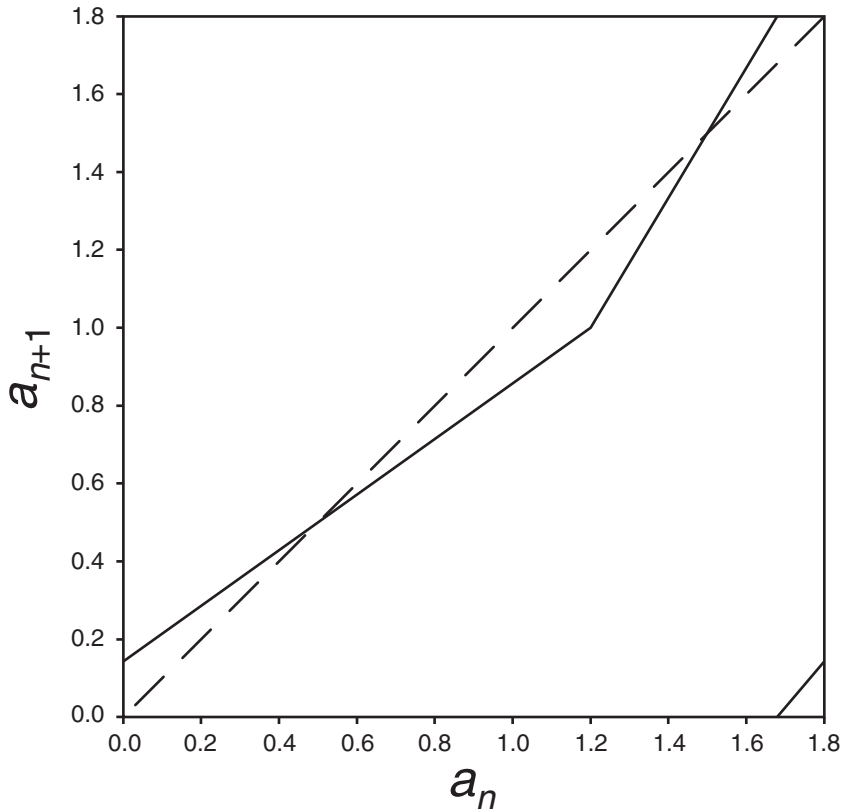

Figure 5.5 Graph of a cell's age after one period of forcing as a function of the cell's age before one period of forcing. The dashed diagonal line is the identity map, $a_{n+1} = a_n$.

is particularly well established for the budding yeast *Saccharomyces cerevisiae* and related species as documented by many experimental studies [7, 8, 11, 18, 19, 26, 31, 38, 58, 63, 64, 65, 68, 91, 97, 98, 99, 101, 102] and numerous models, both distributed [38, 49, 53, 85] and segregated [6, 16, 17, 41, 45, 46, 69, 90, 103, 106, 107] that have been proposed as explanations of the oscillations.

Any cell population for which the distribution of states is not at steady state can be thought of as partially synchronized in the sense that the specific rate of cell division is not constant but changes with a period roughly equal to the length of the cell cycle. In turn, the periodic changes in specific division rate may induce periodic changes in the environment, the medium composition in particular, also with a period roughly equal to the length of the cell cycle. As shown in the previous section, periodic changes in the environment can induce cell cycle synchrony if the period of the changes is of approximately the

same length as the duration of the cell cycle. It is therefore natural to ask if the changes in substrate concentrations that are caused by a partially synchronized cell population can stabilize the synchrony and prevent its decay. If so, the culture would exhibit autonomous oscillations corresponding to periodic solutions to the population balance equations.

We use a simple population balance model of a binary fission organism to show the existence of such periodic solutions, and we will determine the operating conditions, i.e., the values of the dilution rate and substrate feed concentration, at which the solutions are physically meaningful. The model we will analyze is the age distribution PBE with the following additional model equations.

Substrate balance:
$$\frac{dC_S}{dt} = D\ (C_{Sf} - C_S) - \int_0^{a_d} \kappa\, W(t, a)\ da$$

This is probably the simplest possible substrate balance we can pick. The parameter κ is assumed constant and the rate of substrate consumption is therefore proportional to the cell number. Consequently, the rate of substrate consumption will increase rapidly as a cohort of synchronous cells divide, giving rise to clear periodic changes in the substrate concentration.

Model of division age as a function of substrate concentration:
$$a_d = a_d(C_S(t)) = \pi_0 + \frac{\pi_1}{C_S(t-\tau)}$$

In this model equation the parameters π_0, π_1 and τ are assumed constant. This expression for the division age is chosen because it is a simple expression with the expected qualitative behavior: a decrease in division age with an increase in the concentration of the growth-limiting substrate. To reflect the fact that cell metabolism cannot adjust instantaneously to changes in the environment, but requires some amount of time to adjust to new growth conditions, the delay τ is introduced. Thus, the division age at time t is modeled as dependent on the substrate concentration at the previous time $t - \tau$.

For comparison purposes, it will be useful to have the steady-state, nonoscillatory solution. It is given by
$$W(a) = \frac{2}{\kappa} D^2 \left(C_{Sf} - \frac{\pi_1 D}{\ln(2) - \pi_0 D} \right) e^{-Da}$$

and

$$C_S = \frac{\pi_1 D}{\ln(2) - \pi_0 D}$$

from which the washout dilution rate is found to be

$$D_w = \frac{\ln(2)}{\pi_0 + \pi_1 / C_{Sf}}$$

The analysis now proceeds by first postulating a periodic, perfectly synchronized population of cells as a solution for the distribution of states. Using this postulate, we will obtain the expression for the substrate concentration and the age at division versus time. This complete solution will then be checked against the various requirements it must satisfy in order to find the operating conditions over which the solution is valid. The postulated solution for the age distribution has the form

$$W(a, t) = N_0 \, \delta \, (t - a) e^{-Dt}, \quad t \in]0, P]$$

where the solution is periodic with period P and thus $W(a, t) = W(a, t + nP)$ for any integer value of n. The period P and the cell number concentration N_0 are both unknowns at this point, but their values will be determined later in the analysis. Substituting this expression for $W(a, t)$ into the substrate balance gives

$$\frac{dC_S}{dt} = D \, (C_{Sf} - C_S) - \kappa \, N_0 e^{-Dt}, \quad t \in]0, P]$$

which must be solved subject to the boundary condition that the solution is periodic with period P, i.e., $C_S(0) = C_S(P)$, giving

$$C_S(t) = C_{Sf} + \kappa \, N_0 P \frac{e^{-Dt}}{1 - e^{D \, P}} - \kappa \, N_0 t e^{-Dt}, \quad t \in]0, P]$$

from which $a_d(t)$ is readily obtained. When using this result in the following, we must be careful to use it correctly. The expression above is valid only for times between 0 and P, and if the substrate concentration is desired at any other time, one must use the periodicity of $C_S(t)$ to switch the argument into the valid range.

We now apply the cell balance at division. The number of cells immediately after division equals twice the number of cells immediately before division, or

$$N_0 = 2 N_0 e^{-DP} \Rightarrow DP = \ln(2)$$

When this result for P is substituted into the result for the substrate, the following simplified expression is obtained:

$$C_S(t) = C_{Sf} - \left(t + \frac{1}{D}\ln(2)\right) \kappa\, N_0 e^{-Dt}, \quad t \in]0,\ P]$$

Finally, we need to find an expression for the unknown cell number concentration N_0. This is done by making use of the fact that divisions all occur at the age P and at the time equal to P:

$$P = \pi_0 + \frac{\pi_1}{C_S(P - \tau)} \Rightarrow C_S(P - \tau) = \frac{\pi_1}{P - \pi_0}$$

This result is then substituted into the expression for the substrate concentration, giving

$$\frac{\pi_1}{P - \pi_0} = C_{Sf} - (P - \tau + P)\, \kappa\, N_0 e^{-D(P - \tau)}$$

which is combined with the expression for P found above and solved for N_0 to give

$$N_0 = 2D \frac{C_{Sf} - \dfrac{\pi_1 D}{\ln(2) - \pi_0 D}}{(\ln(4) - \tau D)\, \kappa\, e^{D\tau}}$$

The oscillatory solution is summarized as follows:

$$W(a,\ t) = 2\ D \frac{C_{Sf} - \dfrac{\pi_1 D}{\ln(2) - \pi_0 D}}{(\ln(4) - \tau D)\, \kappa\, e^{D\tau}}\, \delta\,(t - a) e^{-Dt}, \quad t \in]0,\ P]$$

$$C_S(t) = C_{Sf} - \left(t + \frac{1}{D}\ln(2)\right) \kappa\, N_0 e^{-Dt}, \quad t \in]0,\ P]$$

$$a_d(t) = \begin{cases} \pi_0 + \dfrac{\pi_1}{C_S(P + t - \tau)}, & 0 < t < \tau \\ \pi_0 + \dfrac{\pi_1}{C_S(t - \tau)}, & \tau < t < P \end{cases}$$

$$P = \frac{\ln(2)}{D}$$

The next step is to check this solution against the constraints that substrate concentration and cell number concentration are positive and that division occurs while a_d is a decreasing function of time. First note

that, as the solution we are investigating is periodic with period P, all values of $\tau + nP$ give the same result for any integer value of n and we need only consider values of τ in the interval from 0 to P. Thus $D\tau < \ln(2) \approx 0.69315\cdots$. Another constraint is obtained by noting that a_d must equal P at some points in time, and therefore $P > \pi_0$, or $D\pi_0 < \ln(2)$.

Checking first that a_d is a decreasing function during cell division, we find

$$\left(\frac{da_d}{dt}\right)_{t=P} < 0 \Rightarrow \left(-\frac{\pi_1}{C_S(t-\tau)^2}\frac{dC_S(t-\tau)}{dt}\right)_{t=P} < 0 \Rightarrow \left(\frac{dC_S(t-\tau)}{dt}\right)_{t=P} > 0$$

and

$$\frac{dC_S(t-\tau)}{dt} = \kappa N_0 e^{-D(t-\tau)}(D(t-\tau)+\ln(2)-1)$$

Evaluating this expression at $t = P$ gives

$$\kappa N_0 e^{-D(P-\tau)}(D(P-\tau)+\ln(2)-1) > 0 \Rightarrow$$
$$D\tau < \ln(4) - 1 \approx 0.38629\cdots$$

Notice that this is a more restrictive bound on $D\tau$ than the one obtained just above.

Next check that the cell number concentration is positive:

$$N_0 > 0 \Rightarrow 2\ D\frac{C_{Sf} - \dfrac{\pi_1 D}{\ln(2)-\pi_0 D}}{(\ln(4)-D\tau)\ \kappa e^{D\tau}} > 0$$

The constraint we just obtained on $D\tau$ assures that the denominator is positive. Thus

$$N_0 > 0 \Rightarrow C_{Sf} > \frac{\pi_1 D}{\ln(2)-\pi_0 D} \quad \text{or} \quad D < \frac{\ln(2)}{\pi_0 + \pi_1 / C_{Sf}} = D_w$$

where obtaining the second inequality from the first required use of the previously derived constraint that $D\pi_0 < \ln(2)$.

The requirement that the substrate concentration is positive is a bit more cumbersome to investigate. We will start by finding the time at which $C_S(t)$ attains its minimum value:

$$\frac{dC_s}{dt} = 0 \Rightarrow$$

$$0 = -\kappa N_0 e^{-Dt_{\min}} + (t_{\min} + \ln(2)/D)\,\kappa N_0 D e^{-Dt_{\min}} \Rightarrow$$

$$t_{\min} = \frac{1}{D}(1 - \ln(2))$$

Notice that $t_{\min}$ is always between 0 and P. The minimum value of C_S is

$$C_S(t_{\min}) = C_{Sf} - \frac{2\kappa N_0}{De}$$

so in order to have a non-negative substrate concentration at all times, we require that

$$C_{Sf} > \frac{2\kappa N_0}{De} = \frac{2\kappa}{De} 2\ D \frac{C_{Sf} - \frac{\pi_1 D}{\ln(2) - \pi_0 D}}{(\ln(4) - \tau D)\ \ \kappa e^{D\tau}}$$

giving

$$\left(\frac{e^{D\tau + 1}}{4}(\ln(4) - D\tau) - 1\right) C_{Sf} + \frac{\pi_1 D}{\ln(2) - \pi_0 D} > 0$$

To evaluate this inequality, note that the coefficient multiplying C_{Sf} is zero for $D\tau = \ln(4) - 1$ and negative elsewhere. But we already require that $D\tau < \ln(4) - 1$, so the coefficient is negative over the range of interest and the inequality becomes

$$C_{Sf} < \frac{\frac{\pi_1 D}{\ln(2) - \pi_0 D}}{1 - \frac{e^{D\tau + 1}}{4}(\ln(4) - D\tau)}$$

The constraints on the oscillatory solution are summarized below:

$$\frac{\pi_1 D}{\ln(2) - \pi_0 D} < C_{Sf} < \frac{\frac{\pi_1 D}{\ln(2) - \pi_0 D}}{1 - \frac{e^{D\tau + 1}}{4}(\ln(4) - D\tau)}$$

$$D\tau < \ln(4) - 1$$

$$D\pi_0 < \ln(2)$$

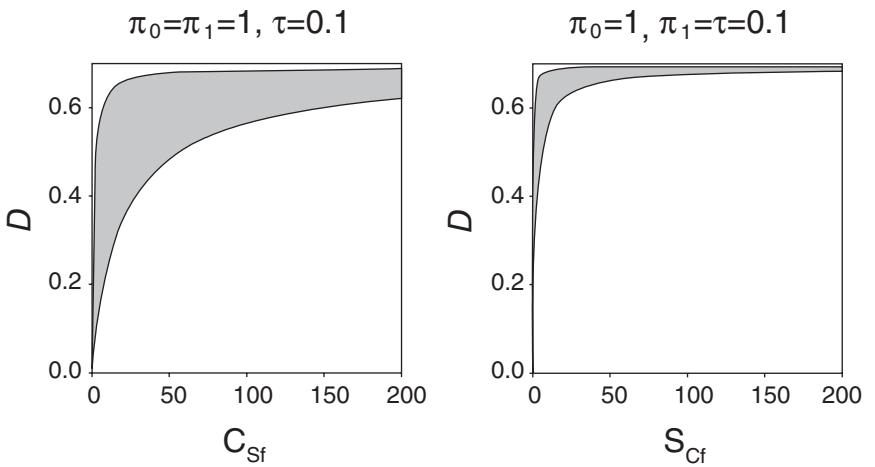

Figure 5.6 Operating diagram showing (gray area) where the autonomously oscillating solution to the PBM satisfies the physical constraints.

These constraints are illustrated by the operating diagrams shown in Fig. 5.6. The gray areas indicate where the constraints are satisfied. The upper boundary of this area is the washout dilution rate. The difference between the two figures is only in the value of the parameter π_1, which changes by an order of magnitude between the two diagrams. As the value of π_1 decreases, so does the effect of changes in substrate concentration on division age, and one would therefore expect less of a tendency to oscillate. This is reflected by the shrinking of the domain over which the physical constraints are satisfied.

It must be emphasized that the analysis above shows only that a periodic solution to the PBM does exists. The more difficult problem of determining whether or not this solution is stable, and therefore experimentally observable, has not been addressed. However, the general problem of determining the stability and bifurcation properties of population balance models is very difficult and still awaits a solution.

5.3 Problems

5.1. Consider the model of autonomous oscillations in a binary fission organism, Sec. 5.2. Find the solution of this model for oscillations with a period half that of the cell cycle length with two identical but out-of-phase subpopulations of synchronized cells and determine the dilution rates and substrate feed concentrations over which the solution is physically valid. The desired solution is illustrated qualitatively in Fig. 5.7.

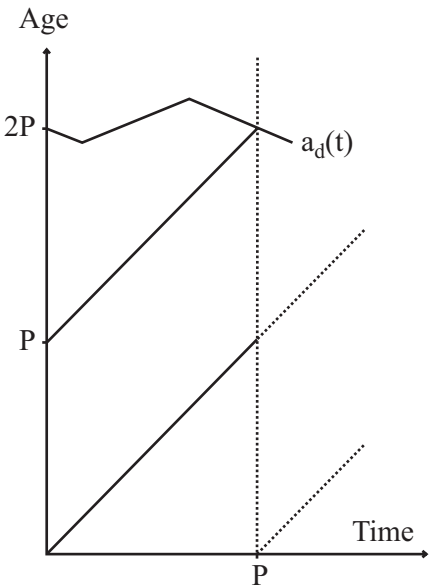

Figure 5.7 Qualitative diagram of division age and growth curves in the time-age plane of an autonomously oscillating culture with two identical but out-of-phase synchronized subpopulations.

5.2. Explore, both by solving the population balance and by finding the iterated map of cell ages, the fate of a population of cells subjected to the forcing in division age of the form

$$a_d(t) = \begin{cases} 2.2 + 0.4t, & 0 < t < 1 \\ 2.6 - 0.4(t-1), & 1 < t < 2 \\ a_d(t-2), & t > 2 \end{cases}$$

5.3. Explore, both by solving the population balance and by finding the iterated map of cell ages, the fate of a population of cells subjected to the forcing in division age of the form

$$a_d(t) = \begin{cases} 1.4 + 0.4t, & 0 < t < 1 \\ 1.8 - 0.4(t-1), & 1 < t < 2 \\ a_d(t-2), & t > 2 \end{cases}$$

Chapter

6

Growth by Branching

There are a large number of organisms that do not grow by a pattern similar to the simple cycle of cell growth followed by a division yielding two new cells. In filamentous organisms such as molds and in root cultures of higher plants, growth occurs by elongation and branching. New cells and/or biomass is formed primarily in the tip or apical region, and formation of lateral branches occurs in a region behind the tip of the parent branch or, in fungal hyphae, by bifurcation of the parent tip. In roots of higher plants, cells that leave the tip cease to divide and undergo a process known as terminal differentiation, in the process forming the structures that make up the root. Thus, cell age in a root equals zero in the tip and increases with distance from the tip. It is thus reasonable to characterize the state of a root by its age distribution and to model the dynamics of the distribution by using some model of the branching kinetics. Several PBMs developed along these lines have been proposed in the literature [36, 51, 56].

The situation for filamentous fungi is somewhat different. The hyphae of higher fungi consist of individual cells, while in lower fungi the cell walls or septa break down and the hyphae consist of a multinucleate branching tube without clearly defined individual cells. Growth occurs primarily from the tip and elongation, and tip bifurcation is usually assumed to be controlled by a cell organ known as the Spitzenkörper [80]. Thus, in spite of the lack of clearly identifiable, individual cells, one may still model growth of the hyphae by a branching model similar to that for roots.

6.1 Branching Rules

We will develop a model of the age distribution in a filamentous organism based on the simple picture of the root or of a filamentous fungi in which the organism is made of well-defined cells with cell ages that increase with distance from the tip or apical region. New cells are formed only in the tip or when a new tip is created during formation of a new lateral branch. New cells formed in the tip leave the tip, cease to divide, and form the body of the root or hyphae. It is the age distribution of these nondividing cells that will be modeled below. It is important to understand that the model is for the age distribution of a single "individual," a single root mat or fungal pellet. We will also assume that the mat or pellet does not break into smaller pieces, although the issue of breakage is immaterial if the age distribution in the model is thought of as the age distribution of the combined pieces. However, if there is no breakage of the pellet, then it is not possible to grow the pellet in a chemostat at steady state, and the model we are considering is therefore for a single pellet in batch. Furthermore, we will assume, at least initially, that growth is not substrate limited; i.e., the model is conceptually similar to that of exponential batch growth of a dividing organism.

The renewal equation for the age distribution balance is

$$W(t, 0) = CT(t)$$

where C is the rate of new cell formation in a tip and $T(t)$ is the number of tips at time t. It follows that the age distribution is

$$W(a, t) = \begin{cases} W_0(a - t), & a > t \\ CT(t - a), & a < t \end{cases} \tag{6.1}$$

where $W_0(a)$ is the distribution at time 0. Clearly, the main problem will be finding $T(t)$, the number of tips as a function of time.

Growth by branching cannot be described by a single characteristic time, such as the length of the cell cycle, but requires several times or delays: the delay between formation of a new branch and formation of the first lateral on this parent branch, the delays between formation of sibling branches, etc. One can describe tip formation dynamics by a set of *branching rules*, verbal statements such as: "the first lateral branch in generation n forms when the parent branch, generation $n - 1$, is 12 hours old," "the second lateral branch in generation n forms 6 hours after the appearance of the first lateral branch in generation n," and so on. Branching rules and the effect of branching on growth can be significantly more complex than these examples indicate. For instance, in roots tips may die, causing loss of apical dominance and

formation of a burst of new laterals [32], and in fungal hyphae the tip extension rate can decrease during apical branching [20]. Branching rules are equivalent to the statements one uses to describe a cell cycle. But a cell cycle is such a simple mode of growth relative to branching that, for the purpose of population balance modeling, we can often describe it just by keywords such as "binary fission." Either way, clearly stated branching rules, or the cell cycle rules, allow us to write cell balances at control points. In the simple model of branching outlined above, the branching rules specify when new tips form, i.e., when cells of age zero appear, and the cell balances are therefore instances of the renewal equation. For instance, if a branching rule states that the *first* tip in a new generation of lateral branches forms on a parent branch the period Δa after the appearance of the tip of the parent branch, then the number of new tips of this type that form at time t, call this number $T_A(t)$, must be equal to the total number of new tips formed at time $t - \Delta a$. Written as a formal equation, this becomes

$$T_A(t) = T(t-\Delta a)$$

where $T(t)$ is the total number of new tips formed at time t. This tip number balance relates the number of tips *of a given type* (in this case tips that are the oldest in their generation) to the number of tips of another type (in this case all extant tips). In general, the tip number balances can be written only after the tips have been classified into such different types. This classification is the only part of the modeling process that requires one to be a bit clever, but for a clearly stated set of branching rules, it is usually not hard to come up with a tip classification which will work.

After all possible tip balances have been written, they must be converted to difference equations. To do so, one must identify a time step or interval Δ such that all the time delays that appear in the branching rules are integer multiples of this Δ. The problem of finding this Δ, given some set of branching delays, is almost identical to the problem of finding an age interval that describes the domain size in the periodic tiling that appears in state space after the age control points become fixed (see Fig. 4.16). Thus, if a branching process is charaterized by the delays Δa_1 through Δa_N, then the times at which new tips form must equal a sum of integer multiples of these delays, i.e.,

$$t_{\text{tipformation}} = \sum_{n=1}^{N} M_n \Delta a_n = \Delta \sum_{n=1}^{N} M_n K_n$$

where Δ is the largest time interval such that all the delays are integer multiples of this interval, $\Delta a_n = K_n \Delta$. This Δ is the time step that will

be used to convert the tip number balances to difference equations. For instance, in the example above, if $\Delta a = K\Delta$, the difference equation version of the tip balance becomes

$$T_A(t = n\,\Delta) = T(n\,\Delta - K\,\Delta) \Rightarrow T_{A,\,n} = T_{n-K}$$

After this is done, either the set of difference equations can be manipulated by standard methods to obtain a single higher-order difference equation for the number of new tips or the coupled difference equations can be solved directly as an instance of a first-order vector difference equation, as in Eq. (4.12). Initial conditions for either equation can be specified from inspection of the physical problem and a well-defined mathematical problem is obtained. Let the difference equation so obtained be

$$\sum_{n=0}^{N} a_n T_{\text{new},\,n+\tau} = 0$$

where a_n is a constant, independent of n. This is a linear, Nth-order difference equation. The parameter τ can be any integer and reflects the fact that the difference equation is invariant to translations by any number of steps along the time axis. (This is similar to an ordinary differential equation of a variable with respect to time. If time does not occur explicitly in the differential equation, then the equation is invariant with respect to translations along the time axis and the origin of the time axis can be chosen arbitrarily.) The dependent variable is the number of new tips as a function of n, the number of time steps of size Δ since time zero. We would like to find the total number of tips T_n, however. Assuming no tip death, the total number of tips is simply

$$T_{n+\tau} = T_{\text{new},\,n+\tau} + T_{n+\tau-1} \Rightarrow T_{\text{new},\,n+\tau} = T_{n+\tau} - T_{n+\tau-1}$$

Substituting this expression into the difference equation for $T_{\text{new},n}$ gives

$$0 = \sum_{n=0}^{N} a_n T_{n+\tau} - \sum_{n=0}^{N} a_n T_{n+\tau-1} \Rightarrow$$

$$\sum_{n=0}^{N} a_n T_{n+\tau} = \sum_{n=0}^{N} a_n T_{n+\tau-1} = \sum_{n=0}^{N} a_{n+} T_{n+\tau-k}$$

where the last equality follows from repeated use of the first equality. The parameter k is any integer, so the only way the last equality can hold is for the sum to be a constant C:

$$\sum_{n=0}^{N} a_n T_{n+\tau} = C \tag{6.2}$$

The total number of tips therefore satisfies a difference equation that, except for the inhomogeneous constant term, is identical to the difference equation for the new tips. The value of the constant will depend on the initial condition, and in the following we will assume that it equals zero. In many instances with biologically reasonable initial conditions, the constant will in fact be identically equal to zero and even when it is not, assuming so does not give rise to a significant error, as we will shortly show.

It is at this point perhaps prudent to recall the theory of linear difference equations: The complete solution to a linear difference equation (constant coefficients not required) is the sum of the homogenous solution and a particular solution. Furthermore, the homogeneous solution is a linear combination of N basis solutions, where N is the order of the difference equation. A particular solution to Eq. (6.2) is a constant, $T = C/\sum_{n=0}^{N} a_n$. Therefore, the solution for the total tip number equals the homogeneous solution, which is identical to the solution for the new tip number, plus this constant. The total tip number will increase without bounds as time increases and the particular solution, the constant solution, will therefore become insignificant relative to the homogenous solution as time becomes large. It is for this reason that it is almost always acceptable to assume that the right-hand side in Eq. (6.2) is zero.

To find the solution for the total number of tips, one needs to find N basis solutions to Eq. (6.2). Fortunately, this is quite easy for an equation with constant coefficients. Assume a solution of the form

$$T_n = \lambda^n$$

and substitute this solution into the difference equation. After simplifications, one obtains the so-called characteristic polynomial of the equation:

$$\sum_{n=0}^{N} a_n \lambda^n = 0$$

and the guessed solution is valid if and only if λ is a root of the characteristic polynomial. We will refer to this polynomial as the branching polynomial. If all the roots of the branching polynomial, say λ_1 through λ_N, have multiplicity one, then they provide the N linearly independent basis solutions needed and the complete solution becomes

$$T_n = C_1\lambda_1^n + C_2\lambda_2^n + \cdots + C_N\lambda_N^n$$

where the C_n's are arbitrary constants that can be determined from the initial conditions. If the characteristic polynomial has multiple roots, additional solutions must be found to obtain a complete set of basis solutions. It can be shown, rather easily in fact, that if a root λ has multiplicity M, a set of basis solutions that correspond to the root is λ^n, $n\lambda^n, n^2\lambda^n, \ldots, n^{M-1}\lambda^n$. We can summarize these results in the closed-form solution

$$T_n = \sum_{r=1}^{\tilde{N}} \left(\sum_{m=1}^{M_r} C_{n,\,m} r^{m-1} \lambda_r^n \right)$$

where $\tilde{N}$ is the number of discrete roots and M_r is the multiplicity of the rth root, λ_r.

Once the T_n's are known, we can go back to the expression for the age distribution, Eq. (6.1). Since $W(a, t)$ is equal to C times the total number of tips at time $t - a$, or $CT_{n = \text{Int}((t-a)/\Delta)}$, where Int( ) is the integer obtained by truncation of the argument, we can write

$$W(a,\,t) = \begin{cases} W_0(a-t), & a > t \\ C \sum_{n=0}^{\text{Int}\,(t/\Delta)} T_n U_n(a,\,t), & a < t \end{cases}$$

where $U_n(a, t)$ equals 1 for $a \in [t - (n+1)\Delta,\ t - n\Delta]$ and zero elsewhere. The zeroth moment, or total number of cells, becomes

$$N(t) = N(0) + C\Delta \sum_{n=0}^{\text{Int}\,(t/\Delta)-1} T_n + C(t - \text{Int}\,(t/\Delta))T_{\text{Int}\,(t/\Delta)}$$

Example 6.1: Branching rules with two delays Consider a filamentous organism that grows according to the following branching rules: (1) The first lateral branch in a new generation is formed on the parent branch a time period $a_b + \Delta a$ after the parent branch first appeared. (2) Once started, formation of lateral branches continues in such a way that the delay between initiation of new lateral branches equals Δa.

The structure of an organism growing by these branching rules is sketched in Fig. 6.1. One can classify the new tips by their number in the sequence of siblings, but this does not work well because it creates an infinity of tip types. Instead, note that tips can form either as the first tip on a branch, the event marked A or as one of the later tips, the event marked B. If $T_A(t)$ denotes the number of new tips of type A, $T_B(t)$ the number of new tips of type B and $T(t)$ the total number of new tips, then the tip balances are

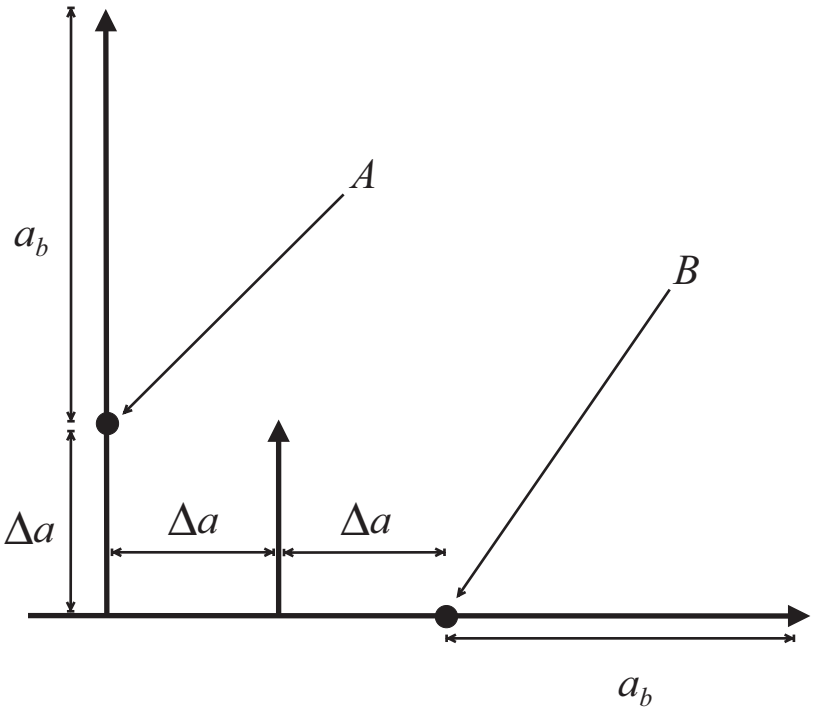

Figure 6.1 Branching rules with two delays. The first lateral branch that forms on a parent branch is indicated by the event *A*, while subsequent branching events are indicated by *B*.

$$T_A(t) = T(t - a_b - \Delta a), \qquad \text{Rule 1}$$
$$T_B(t) = T(t - \Delta a), \qquad \text{Rule 2}$$

So the equation for the total number of new tips formed at time t is

$$T(t) = T_A(t) + T_B(t)$$

Let $\Delta a = K\,\Delta$ and $a_b = L\,\Delta$. The difference equations become

$$T_{A,\,n} = T_{n-L-K}$$
$$T_{B,\,n} = T_{n-K}$$
$$T_n = T_{A,\,n} + T_{B,\,n}$$

and hence

$$T_n = T_{n-K} + T_{n-L-K}$$

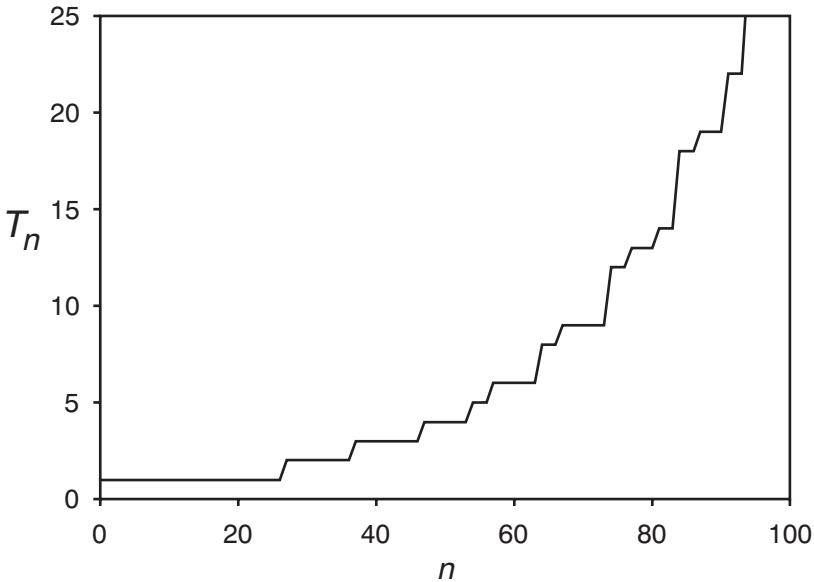

Figure 6.2 Total number of tips for branching rules with two delays, $K = 10$ and $L = 17$.

or equivalently

$$T_{n+K+L} - T_{n+L} - T_n = 0$$

with a similar equation holding for the total tip number.

The initial condition for this difference equation must specify values of T_0 through T_{K+L-1}, and the values must be chosen so that they make sense biologically. For instance, they cannot decrease with time and they certainly cannot be negative. To get sensible values let the inoculum be a branch formed from a tip that appeared at time 0. This branch will form its first lateral at time $a_b + \Delta a$, or after $L + K$ time steps. Thus, $T_{\text{new},0} = 1$ and $T_{\text{new},1}$ through $T_{\text{new},K+L-1}$ equal 0. Or, considering the total number of tips, T_0 through T_{K+L-1} equals 1. As the first lateral branch forms after $K + L$ time steps, $T_{\text{new},K+L} = 1$ and $T_{K+L} = 2$. The constant C in Eq. (6.2) is therefore identically zero. With this initial condition, the solution for T_τ can be found for any values of K and L. The solution is plotted in Fig. 6.2 for $K = 10$ and $L = 17$.

More interesting perhaps than the total tip number is the total cell number, or the zeroth moment of the age distribution N_n. This is shown in Fig. 6.3.

The growth curve exhibits an initial transient during which the specific growth rate is decreasing, qualitatively the opposite of the classical lag phase. A classical lag is not expected since the model assumes no substrate limitation but the opposite type of transient requires an explanation. It is seen because the initial condition, or inoculum, is a tiny segment of a branch

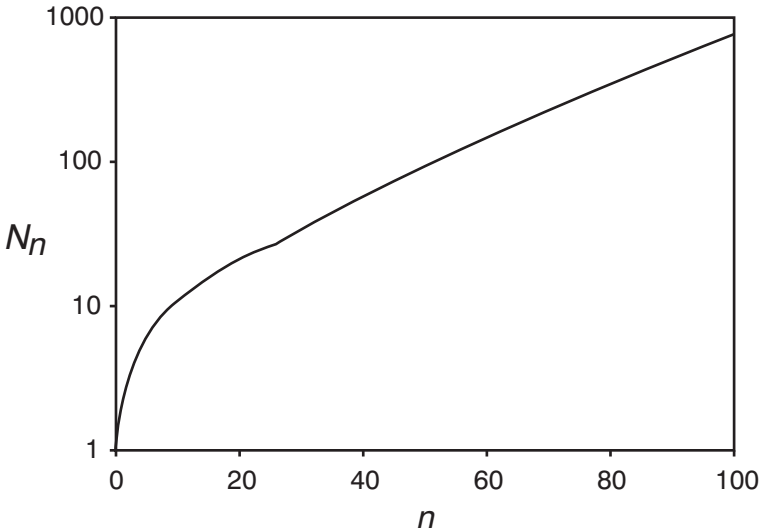

Figure 6.3 Total number of cells for branching rules with two delays, K =10 and L = 17.

close to the tip, i.e., a segment from which cells that are not yet old enough to form laterals have been removed. Removal of these unproductive cells does not change the growth rate or tip formation rate, but it does lower the total amount of cells, thus increasing the *specific* growth rate. In fact, for the hypothetical inoculum used in this example, the cell number at time zero is zero and the specific growth rate at time zero must therefore equal infinity, explaining the vertical tangent to the growth in the Fig. 6.3 curve at $t = 0$. Evidently, the initial transient is more an artifact of the model idealizations than a measurable phenomenon.

The example above shows that the shape of the age distribution of a branching organism changes continuously and so does its specific growth rate. A growth curve will exhibit an initial transient caused by the transient in the branching dynamics, and this transient is phenomenologically different from the classical lag phase caused by transient adaptation of the inoculum to the fresh medium. However, a limiting value of the specific growth rate is reached as time goes to infinity, and, from an engineering perspective, the specific growth rate may reach this limiting value early on in a growth curve. The limiting specific growth rate is by definition

$$\mu = \lim_{t \to \infty} \frac{1}{t_2 - t_1} \ln \left(\frac{\int_0^\infty W(t_2, a) da}{\int_0^\infty W(t_1, a) da} \right)$$

Assuming that the root of the characteristic polynomial with the numerically largest real part, λ_P, has an algebraic multiplicity of 1, it is not hard to show that [51]

$$\mu = \frac{1}{n_2 \Delta - n_1 \Delta} \ln \left(\frac{\lambda_P^{n_2}}{\lambda_P^{n_1}} \right) = \ln (\lambda_P) / \Delta$$

where $t_2 = n_2 \Delta$ and $t_1 = n_1 \Delta$. The result is this simple only when λ_P has multiplicity 1. Clearly, it is λ_P, the root of the characteristic polynomial with the numerically largest real part, that is of primary interest in these models because it is this root that determines the specific growth rate in the culture at all but the earliest times. Luckily, for most realistic branching rules, this polynomial root turns out to be easy to find. Most realistic branching rules give characteristic polynomials of the form

$$\lambda^N - \sum_{n=0}^{N-1} a_n \lambda^n \tag{6.3}$$

where a_n is positive. Negative a_n values could conceivably occur if branching events were not independent and new branch formations were inhibited by older branches. For polynomials of this type, the following claim can be shown to hold [51].

Claim

A polynomial of the form in Eq. (6.3) has a unique, positive root of multiplicity 1, and this root is an upper bound on the absolute value of the real part of all other roots.

This result simplifies a numerical search for λ_P.

6.2 Simulation of Tip Numbers

Rather than solve the difference equation for the tip numbers, Eq. (6.2), analytically, a procedure that requires finding the roots of the characteristic polynomial and the values of the arbitrary constants, the equation can be evaluated directly on a computer. One simply uses the initial condition, given as values of T_0 through T_{N-1}, to find T_N as

$$T_N = \frac{C}{a_N} - \frac{1}{a_N} \sum_{n=0}^{N-1} a_n T_n$$

then one replaces the initial condition with the values T_1 through T_N and repeats the process. This evaluation can be done up to large times quite quickly, so rapidly in fact that the trouble of finding the analytical solution does not appear worthwhile, except for the rigorous insight it provides into the solution structure.

However, this still leaves one with the need to derive the difference equation for a given set of branching rules, a tedious and error-prone process. To get around this problem, the tip number can be simulated directly from the branching rules by using a simple algorithm [51]. Although implementation of the algorithm is a bit more involved than direct evaluation of the difference equation, the algorithm has the great advantage that model parameters can easily be changed without changing the code or without the need for a rederivation of a difference equation, thus making it quite easy to explore the effect of changes in model parameters on tip formation kinetics.

In the algorithm, each branch in the root or hyphae is represented by an element of a one-dimensional array or table. The element representing a given branch contains the time at which a new branching event will occur on this branch. Let the initial condition be a single branch with no lateral branches. This initial condition is represented by an array with a single element, $[t_0]$, where the branching time t_0 will depend on the age of the inoculum branch. For the purpose of illustration, assume now that the branching rules are the two rules used in Example 6.1. To start the simulation, the array is scanned to find the time of the first upcoming branching event, call it t_i, and the branch on which this occurs. The value of the element representing the branch on which the event will occur is increased by Δa and the size of the array is increased by 1. The new element represents the new branch, and for the branching rules proposed above, the value of this component must therefore be $t_i + a_b + \Delta a$. After the array has been updated this way it can be scanned again for the next branching event and so forth. This process is illustrated below. After the first lateral branch is formed, the array takes the form

$$\begin{pmatrix} \text{Branch } 1 \\ \text{Branch } 2 \end{pmatrix} = \begin{pmatrix} t_1 = t_0 + \Delta a \\ t_2 = t_0 + \Delta a + a_b \end{pmatrix}$$

and, clearly, the next branching event must take place on branch 1, giving

$$\begin{pmatrix} \text{Branch } 1 \\ \text{Branch } 2 \\ \text{Branch } 3 \end{pmatrix} = \begin{pmatrix} t_1 = t_0 + 2\,\Delta\, a \\ t_2 = t_0 + \Delta\, a + a_b \\ t_3 = t_0 + 2\,\Delta\, a + a_b \end{pmatrix}$$

At this point, the next step can yield two different arrays, depending on the relative magnitude of a_b and Δa. If $t_1 < t_2$ then

$$\begin{pmatrix} \text{Branch } 1 \\ \text{Branch } 2 \\ \text{Branch } 3 \\ \text{Branch } 4 \end{pmatrix} = \begin{pmatrix} t_1 = t_0 + 3\,\Delta\, a \\ t_2 = t_0 + \Delta\, a + a_b \\ t_3 = t_0 + 2\,\Delta\, a + a_b \\ t_4 = t_0 + 3\,\Delta\, a + a_b \end{pmatrix}$$

while, if $t_1 > t_2$,

$$\begin{pmatrix} \text{Branch } 1 \\ \text{Branch } 2 \\ \text{Branch } 3 \\ \text{Branch } 4 \end{pmatrix} = \begin{pmatrix} t_1 = t_0 + 2\,\Delta\, a \\ t_2 = t_0 + 2\,\Delta\, a + a_b \\ t_3 = t_0 + 2\,\Delta\, a + a_b \\ t_4 = t_0 + 2\,\Delta\, a + 2a_b \end{pmatrix}$$

This algorithm is simple to program and can be used for any branching rules that do not exibit any coupling between branching events. In other words, any rules that are local in the sense that the timing for formation of a lateral branch is independent of branching events occurring elsewhere on the root/hyphae. The algorithm is not efficient in the sense that the computational time increases exponentially with the duration of the growth period being simulated. However, execution up to large times is still so fast on a desktop PC that this will almost never be an issue.

A significant advantage of this algorithm over the analytical approach is that stochastic branching times can easily be incorporated. Just as microbial cells have distributed division ages or states, the branching times in roots and hyphae are not constants but vary between branching events. This variation is of less concern in the study of fungi than in the study of roots. Fungal cultures are usually inoculated with a large number of cells, and the law of large numbers assures that replicate experiments have approximately the same

average growth dynamics. Roots, on the other hand, are often studied in small cultures, even as single roots, and stochastic branching times can result in huge variations between roots grown under identical conditions. It is therefore important to understand and quantify this inherent variation and not confuse it with the variation due to different growth conditions.

To illustrate the variation in growth curves caused by stochastic branching parameters, consider the branching rules from Example 6.1, but assume that the two characteristic times a_b and Δa are log-normally distributed stochastic variables. To simulate this situation, the algorithm described above needs only to be supplied with a method of generating log-normal random numbers for a_b and Δa. (Methods for generation of pseudo-random numbers are available in the literature for all commonly used probability distributions, and many of these are included as routines in popular mathematical software packages.) An example of such a simulation is shown in Fig. 6.4. The simulations were done with log-normal distributed values of a_b and Δa with means of 2 and 1 respectively, and a variance of 1 for each, and the graph shows 10 tip number growth curves.

The 10 curves are quite different, and one of them shows much faster initial growth than all the others. This is not due to any inherent

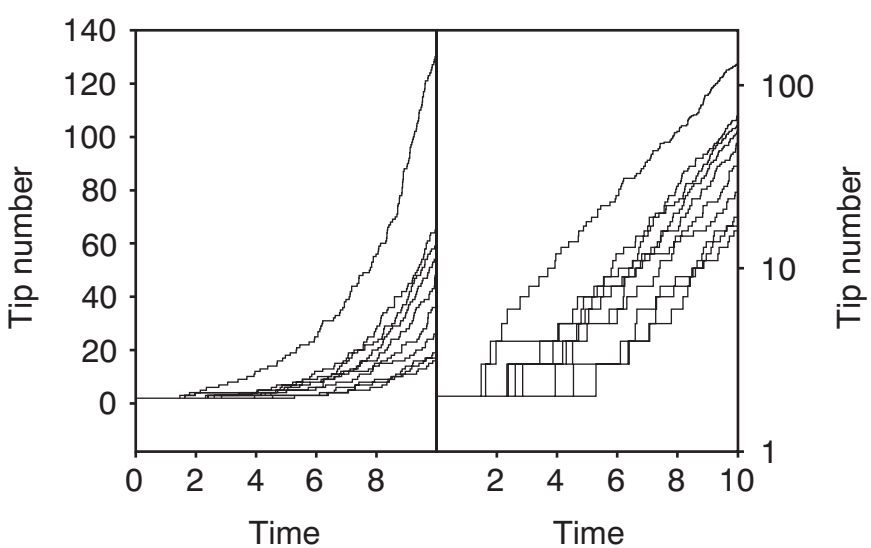

Figure 6.4 Ten tip number growth curves for stochastic branching using the branching rules of Example 6.1. The two branching parameters are log-normally distributed with means $\Delta a = 1$ and $a_b = 2$ and variance 1. The figure of the left shows the growth curves plotted on a linear scale, the figure on the right shows the same curves plotted on a logarithmic scale.

differences between the growth kinetics of the roots, but is caused by the random occurrences of early branching events on the inoculum branch. Similarly, the growth curves that show the lowest initial growth rates do so because the initial branching events occurred late. Given enough time, all the simulated roots develop so many tips that growth becomes insensitive to the randomness of the individual branching events and all roots approach the same limiting specific growth rate, as evidenced by the similar slopes of the asymptotes in the logarithmic plot of the growth curves on the right of Fig. 6.4.

A more detailed description of the variation among tip number growth curves is obtained by running a large number of simulations to find the probability distribution of tip numbers versus time. The results of 20,000 such simulations are shown as a contour plot of the tip number probability distribution in Fig. 6.5. The simulations required for this figure took less that 1 minute on a desktop PC and used the same model parameters as the simulations depicted in Fig. 6.4.

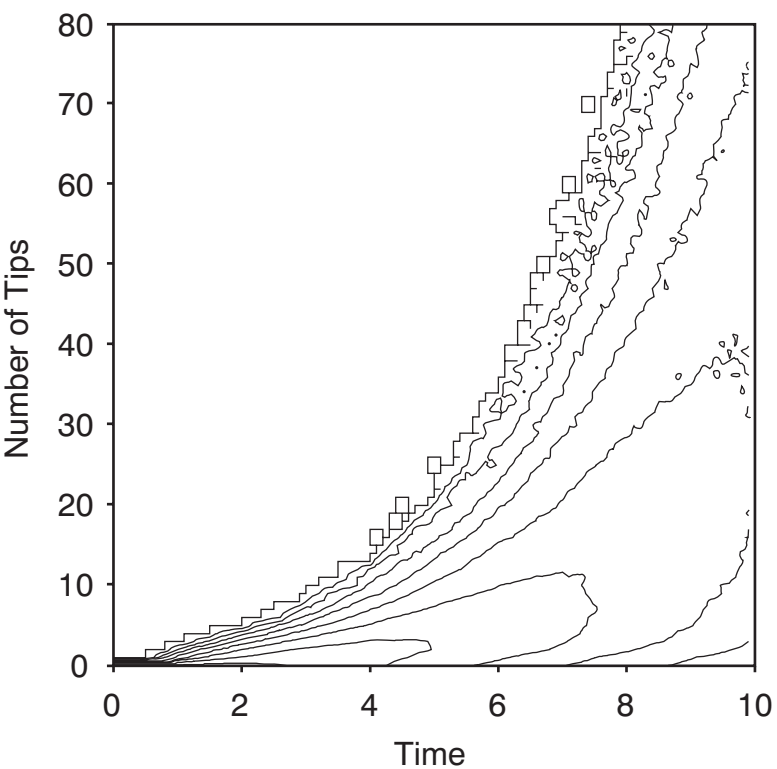

Figure 6.5 Contour plot of the tip number probability distribution versus time using the same model parameters as in Fig. 6.4. Twenty thousand growth curves were simulated to generate this figure.

The plot clearly shows how a well-defined initial condition of a single tip can quickly produce a wide distribution of tip numbers when branching times are not constant.

Another use of the simulation algorithm is to investigate the effect of tip death. Just as individual microbial cells in a culture may die, individual tips in a root or hyphae may also die although the entire root/hyphae dies only if all its tips are dead. Consider, once more, the branching rules from Example 6.1 with constant model parameters, and add a constant probability that a tip can die in each time step. The only change needed in the code is to include a call to a random number generator, once for each tip in each time step, to determine whether or not the tip dies in that time step. The result of such a simulation is shown in Fig. 6.6.

The figure shows a series of seven histograms of the frequency of tip numbers during the first seven time steps. The tall bars indicate the growth of roots or hyphae that have not experienced any tip death and clearly show the approach toward exponential growth. Each of these tall bars has a tail of lower bars at a lower tip frequency, indicating the presence of roots/hyphae on which one or more tips have died. Finally, the ridge of low bars in the front of the figure indicates the

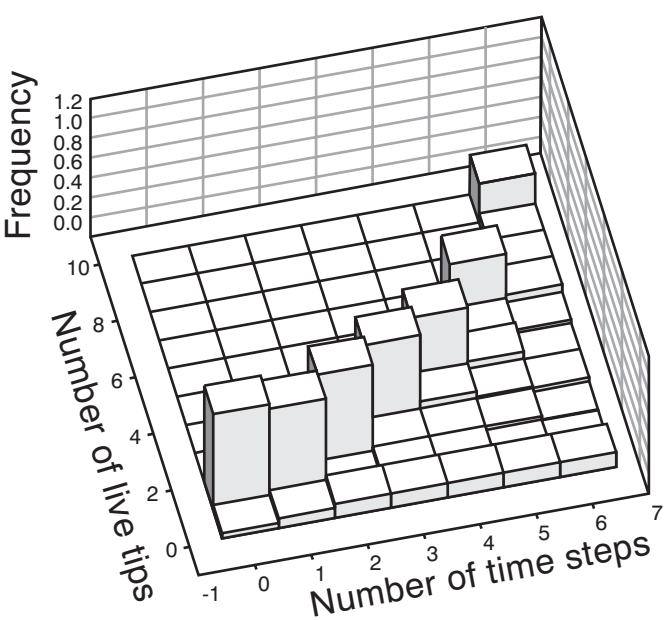

Figure 6.6 Histograms of the number of live tips versus the number of time steps in the simulation. The histograms are the result of 10,000 simulations with a probability that a tip will die in one time step equal to 0.05. The branching rule parameters are $\Delta a = 1$ and $a_b = 2$.

frequency of roots/hyphae that are dead because all tips have died. This frequency increases fast in the beginning when the total tip number is low, but quickly approaches a steady value at large times. The steady value is reached because, once the number of tips on a root/hyphae is reasonably large, the probability that all tips will die becomes vanishingly small. The figure thus shows that in a set of cultures, each using a single tip as inoculum, one can expect that the cultures will partition into two groups, a small group of dead cultures and a larger group of live cultures, most of which have experienced approximately similar specific growth rates.

6.3 Problems

6.1. Find the branching polynomial for the following branching rules.

1. New lateral branches are formed at a point on the root where the cells in the parent branch have reached a fixed age a_b.
2. The first lateral branch is formed when the cells at the base of the parent branch have the age $\Delta\tilde{a} + a_b$.
3. Once started, branching continues in such a way that the distance between branch points equals a constant age difference Δa.

You can assume that $\Delta a < a_b$.

6.2. Find the branching polynomial for the following branching rules.

1. New lateral branches are formed at a point on the root where the cells in the parent branch have reached a fixed age a_b.
2. The first lateral branch is formed when the cells at the base of the parent branch have the age $\Delta a + a_b$.
3. Once started, branching continues in such a way that the distance between branch points equals a constant age difference Δa.
4. Branching stops and the parent tip dies after a total of Z lateral branches have formed on the parent branch.

The death of a tip often results in rapid formation of several lateral branches behind the dead tip. This phenomenon is referred to as loss of apical dominans and can, for instance, be observed when a tip or shoot is cut off a growing plant. Usually new branches will quickly form below the cut. To model the dynamics of this response, find the branching polynomial when the branching rules above are augmented with the following rule.

5. After the last of the Z normal lateral branches have formed, a period $\Delta\tilde{a}$ passes before X additional laterals form as a result of loss of apical dominans.

Chapter

7

Alternative Formulations

An alternative formulation of the population balance distribution in exponential growth was originally derived for the length distribution, $f(l)$ [22, 37]. It is usually called the Collins-Richmond equation,

$$r(l) = \mu \int_0^l (2\psi(\tilde{l}) - \phi(\tilde{l}) - f(\tilde{l}))d\tilde{l} / f(l)$$

where $r(l)$ is the rate of change of cell length l, $\psi(l)$ the probability that a newborn cell has a length between l and $l + dl$, and $\phi(l)$ the probability that a dividing cell has a length between l and $l + dl$. In this form, the equation assumes no cell death, but it can be extended to do so [100]. This equation appears to be substantially different from our previous formulation of a PBE, but can in fact be derived from this [75]. The two formulations use different probability functions, and one must derive equations between these different functions in order to transform one formulation to the other. Doing so is more than an idle exercise in variable transformation and is important because it allows one to find equations between probability functions that can be measured experimentally (at least in theory) and nonmeasurable functions that appear in a given PBM formulation. We will therefore briefly look at the derivation of a few equations between various probability functions. Experimentally obtained distributions, together with a discussion of some of the problems and issues concerning these measurements, are given elsewhere [70, 71].

It is important to understand clearly what the function $\phi(z)$ represents. This function is the distribution of probabilities that a dividing cell has a state between z and $z + dz$; i.e., the function is determined empirically by measuring the state of cells caught in the

act of dividing. We will refer to $\phi(z)$ as the *distribution of division states*. This is different from the *a priori distribution of division states*, the probability that a newborn cell selected at random will have a state between z and $z + dz$ when it divides. We will denote this distribution $h(z)$.

The difference between these two distributions is subtle and requires a brief illustrative example. For instance, if in a population growing at steady state in a chemostat, a newborn cell, picked at random, has a probability of 0.5 of dividing at the age of 20 minutes and an equal probability of dividing at an age of 30 minutes, then the a priori distribution of division states is

$$h(a) = 0.5\delta(a - 20) + 0.5\delta(a - 30)$$

where $\delta(a)$ is the Dirac delta function. However, because the cells that divide at an age of 30 minutes stay longer in the reactor after being born than cells that divide after 20 minutes, more 30-minute cells will wash out before they have a chance to divide than 20-minute cells. Consequently, the probability that a dividing cell, picked at random, will have the age 20 minutes is greater than the probability that it will have the age 30 minutes, and the distribution of division states $\phi(a)$ will therefore be skewed toward younger ages and have the form

$$\phi(a) = A\delta(a - 20) + (1 - A)\delta(a - 30)$$

where A is some probability greater than 0.5. The actual value of A will depend on the dilution rate. The explicit dependence of $\phi(z)$ on dilution rate indicates that it is probably not a good indicator of the physiological state of the cell population. The a priori distribution of states $h(z)$ depends only implicitly on the dilution rate of the reactor and is therefore a better candidate for models of cell division. The distribution of division ages also depends on the age of the cells one is considering. This is seen in human populations, where it is common knowledge that the life expectancy of a newborn child is different from that of an older child. The older child has a higher life expectancy because it has already survived the initial years of life while the newborn child still is at risk for infant mortality. In the example above, the distribution of division ages of cells older than 20 minutes is $\delta(a - 30)$, different from $h(a)$.

Let us first derive an equation between the distribution of division ages, $\phi(a)$, and the a priori distribution of division ages, $h(a)$. We will work directly from the definitions. $\phi(a)$ is defined by

$$\phi(a)da = \text{fraction of dividing cells with age } a$$

but this can be written as

$$\phi(a)da = \frac{\text{number of cells born at time } t-a \text{ with an a priori division age } a}{\text{number of dividing cells}}$$

If we consider a time interval of length Δt, then the number of dividing cells during this interval will equal the net increase in cell number. Thus, the number of dividing cells is $(N(t)e^{\mu\Delta t} - N(t))$, where μ is the specific growth rate of the culture and $N(t)$ is the total cell number at time t.

Similarly, the term in the numerator, the number of cells born in the time interval from $t - a$ to $t - a + \Delta t$, must equal twice the net increase in cell number over this interval, i.e., $2(N(t-a)e^{\mu\Delta t} - N(t-a))$ — twice because the net increase equals the number of divisions and each division creates two new cells (while destroying one old cell). Multiply this number by $h(a)\,da$ to obtain the number of cells born between $t - a$ and $t - a + \Delta t$ with an a priori division age $a \in [a, a + da]$, or $2(N(t-a)e^{\mu\Delta t} - N(t-a))h(a)da$. Putting these expressions into the equation for $\phi(a)$ and simplifying gives

$$\phi(a) = 2e^{-\mu a}h(a)$$

which is the desired equation.

The first moment of the a priori distribution of division ages is the average cell cycle length, or the average doubling time on the single-cell level:

$$< t_{\text{c.c.}} > = \int_0^\infty a h(a) da$$

One might hypothesize that $< t_{\text{c.c.}} > = t_d$, where t_d is the population doubling time, $t_d = \ln 2 / \mu$. Certainly this is true if all divisions occur at the same age, but it turns out not to hold otherwise. To see this, start by noting that the distribution of division ages, $\phi(a)$, obviously satisfies

$$1 = \int_0^\infty \phi\,(a)da$$

Writing $\phi(a)$ in terms of $h(a)$, one obtains

$$1 = \int_0^\infty 2e^{-\mu a}h(a)da = 2e^{-\mu < t_{\text{c.c.}} >} \int_0^\infty e^{-\mu(a - < t_{\text{c.c.}} >)} h(a)da$$

We now use the inequality $1 - x < e^{-x}$, which is valid for all x different from 0. This gives the inequality

$$2e^{-\mu<t_{c.c.}>}\int_0^\infty (1-\mu(a-<t_{c.c.}>))h(a)da<1$$

Clearly,

$$\int_0^\infty (1-\mu(a-<t_{c.c.}>))h(a)\,da =$$

$$\int_0^\infty h(a)\,da-\mu\int_0^\infty ah(a)\,da+\mu<t_{c.c.}>\int_0^\infty h(a)\,da=1$$

so one obtains

$$<t_{c.c.}>>\ln 2/\mu$$

This result was derived by Painter and Marr [67], who also derived an approximate formula for $t_{c.c.}$ in terms of μ and the variance of the a priori distribution of division ages.

We will now derive an equation between $\Gamma(z)$ and $\phi(z)$. Start with the following obviously true equality:

(Rate of cell division) (fraction of dividing cells in state z)
= rate of division of cells in state z

Each factor in this balance is known. The rate of cell division is μN, the fraction of dividing cells in state z is $\phi(z)dz$, and the rate of division of cells in state z is $\Gamma(z)W(z)dz$. Therefore,

$$\Gamma(z)=\frac{\mu\,\phi\,(z)}{f(z)}$$

We can eliminate $f(z)$ from this result by solving the Collins-Richmond equation for $f(z)$ and substituting the solution into the expression above. The Collins-Richmond equation can be solved for the distribution of states by first differentiating the equation with respect to z to get the ordinary differential equation

$$\frac{d}{dz}(f(z)r(z))=\mu(2\psi(z)-\phi\,(z)-f(z))$$

and solving this subject to the boundary condition $f(0)=0$,

$$f(z)=\frac{\mu}{r(z)}\int_0^z \exp\left(\mu\int_z^{\tilde{z}}\frac{d\tilde{\tilde{z}}}{r(\tilde{\tilde{z}})}\right)(2\psi(\tilde{z})-\phi\,(\tilde{z}))d\tilde{z}$$

which finally gives

$$\Gamma(z) = \frac{\phi(z)r(z)}{\int_0^z \exp\left(\mu\int_z^{\tilde{z}} \frac{d\tilde{\tilde{z}}}{r(\tilde{\tilde{z}})}\right)(2\psi(\tilde{z}) - \phi(\tilde{z}))d\tilde{z}}$$

An equation between the a priori distribution of division states and the division intensity is derived as follows: Assuming that the state parameter z increases with time, the probability that a newborn cell will divide before reaching the state z is

$$\int_0^z h(\tilde{z})d\tilde{z}$$

and the probability that a cell which has reached the state z will divide between z and $z + dz$ is therefore[1]

$$\frac{h(z)dz}{1 - \int_0^z h(\tilde{z})d\tilde{z}} = \frac{h(z)dz}{\int_0^\infty h(\tilde{z})d\tilde{z}}$$

The probability of division between z and $z + dz$ equals the probability of division between t and $t + dt$, where dt is given by $dz/dt = r(z)$. But this is exactly how the division intensity was defined, so

$$\Gamma(z)dt = \frac{h(z)dz\frac{dt}{dt}}{\int_z^\infty h(\tilde{z})d\tilde{z}} = \frac{r(z)h(z)dt}{\int_z^\infty h(\tilde{z})d\tilde{z}} \Rightarrow \Gamma(z) = \frac{r(z)h(z)}{\int_z^\infty h(\tilde{z})d\tilde{z}}$$

Notice that for $h(z) = \delta(z - z_d)$, we obtain Eq. (3.1).

Example 7.1: Two discrete division ages We will find the distribution of divisions ages in the case for which the a priori distribution of division ages is given by an expression of the form

$$h(a) = 0.5\delta(a - a_1) + 0.5\delta(a - a_2)$$

For this case, the normalized age distribution has a discontinuity at age a_1, and, assuming without loss of generality that $a_1 < a_2$, the cell balances take the form

[1]The result is obtained by using the conditional probability theorem, typically written $P(A \mid B) = P(A \cap B)/P(B)$, or, stated in words: The probability of A occurring, assuming that B has occurred, equals the probability of A and B occurring divided by the probability of B occurring.

$$f_1(0) = 2 \cdot 0.5 f_1(a_1) + 2 f_2(a_2)$$
$$0.5 f_1(a_1) = f_2(a_1)$$

where $f_1(a) = C_1 \exp(-Da)$ and $f_2(a) = C_2 \exp(-Da)$. Solving for the arbitrary constants and applying the normalization condition gives

$$f(a) = \begin{cases} \dfrac{2D}{2 - \exp(-Da_1) - \exp(-Da_2)} e^{-Da}, & 0 < a < a_1 \\ \dfrac{D}{2 - \exp(-Da_1) - \exp(-Da_2)} e^{-Da}, & a_1 < a < a_2 \end{cases}$$

where the two division ages are bound by the constraint that

$$1 = e^{-Da_1} + e^{-Da_2}$$

The distribution of division ages $\phi(a)$ is now

$$\begin{aligned} \phi(a) &= \frac{(f_1(a_1) - f_2(a_2))\delta(a - a_1) + f_2(a_2)\delta(a - a_2)}{(f_1(a_1) - f_2(a_1)) + f_2(a_2)} \\ &= e^{-Da_1}\delta(a - a_1) + e^{-Da_2}\delta(a - a_2) \\ &= 2e^{-Da} h(a) \end{aligned}$$

which is a special case of the general result derived previously.

7.1 Problems

7.1. Assuming that the distribution of division ages, $\phi(a)$, in a chemostat is

$$\phi(a) = 0.5\delta(a - a_1) + 0.5\delta(a - a_2)$$

find:

A. The age distribution in the culture in terms of the dilution rate

B. a_1 and a_2

C. The equation that the value for the dilution rate must satisfy

You can assume without loss of generality that $a_1 < a_2$.

7.2. The life span of a cell can be defined as the time between formation of the cell and the first subsequent division. With this definition in mind, given the a priori distribution of division ages, $h(a)$, what is the life expectancy of a newborn cell? Find an expression for the life expectancy of a cell that has attained the age a_1.

7.3. The distribution of division ages of extant cells is the distribution obtained by taking cells at random from the culture and measuring their

division ages. Of course, it is not possible to actually do this because one cannot measure the age of a cell picked at random and therefore one cannot determine its division age even if one observes the cell until it divides. Even though the distribution of division ages of extant cells is not a directly measurable distribution, it can be useful in models. To relate this distribution to something measurable, show that it equals

$$2(1 - e^{-\mu a})h(a)$$