14 The Kinetic Sub-model of SSF Bioreactor Models: General Considerations

David A. Mitchell and Nadia Krieger

14.1 What Is the Aim of the Kinetic Analysis?

As pointed out in Chap. 12, a mathematical model of an SSF bioreactor requires two sub-models, a sub-model that describes the growth kinetics of the microorganism and a sub-model that describes the energy and mass balances and transport phenomena. Each of these sub-models is written at an appropriate level of detail, depending on what simplifications and assumptions have been made. Chapter 13 argued for the use of simple empirical equations within the kinetic sub-model, in order not to make it too difficult to solve the bioreactor model. Chapters 14 to 17 address various questions related to the establishment of kinetic sub-models of this type (Fig. 14.1).

The aim is to write a kinetic equation in which the change in the amount of biomass, or a variable associated with it, is described by a differential equation, with the parameters of this differential equation taking into account the effect on growth of the key state variables that will be included in the bioreactor model, such as the temperature and water activity of the substrate bed. This is achieved as shown in Fig. 14.2. Note that the experiments done for the purpose of selecting the kinetic equation should be done after some efforts have been made to find a medium on which the organism grows well and to identify the optimal environmental conditions. This book does not address the optimization of the medium and environmental conditions (see the further reading section at the end of this chapter). A kinetic profile is constructed by measuring the biomass, or some indirect indicator of the biomass, in samples removed over the time course of the fermentation (Fig. 14.2(a)). Various kinetic equations are fitted to the data by regression and the one that fits best to the data is selected. Later, experiments are done in which different environmental conditions are imposed, such that, after analysis of the growth profile in each condition, plots can be made that relate the parameters of the kinetic equation to the environmental variable (Fig. 14.2(b)). Each kinetic parameter will then be expressed as an empirical function of the environmental parameter.

The current chapter covers some of the issues that must be addressed before beginning the process of kinetic modeling and then goes on to explain how the basic kinetic equation is selected.

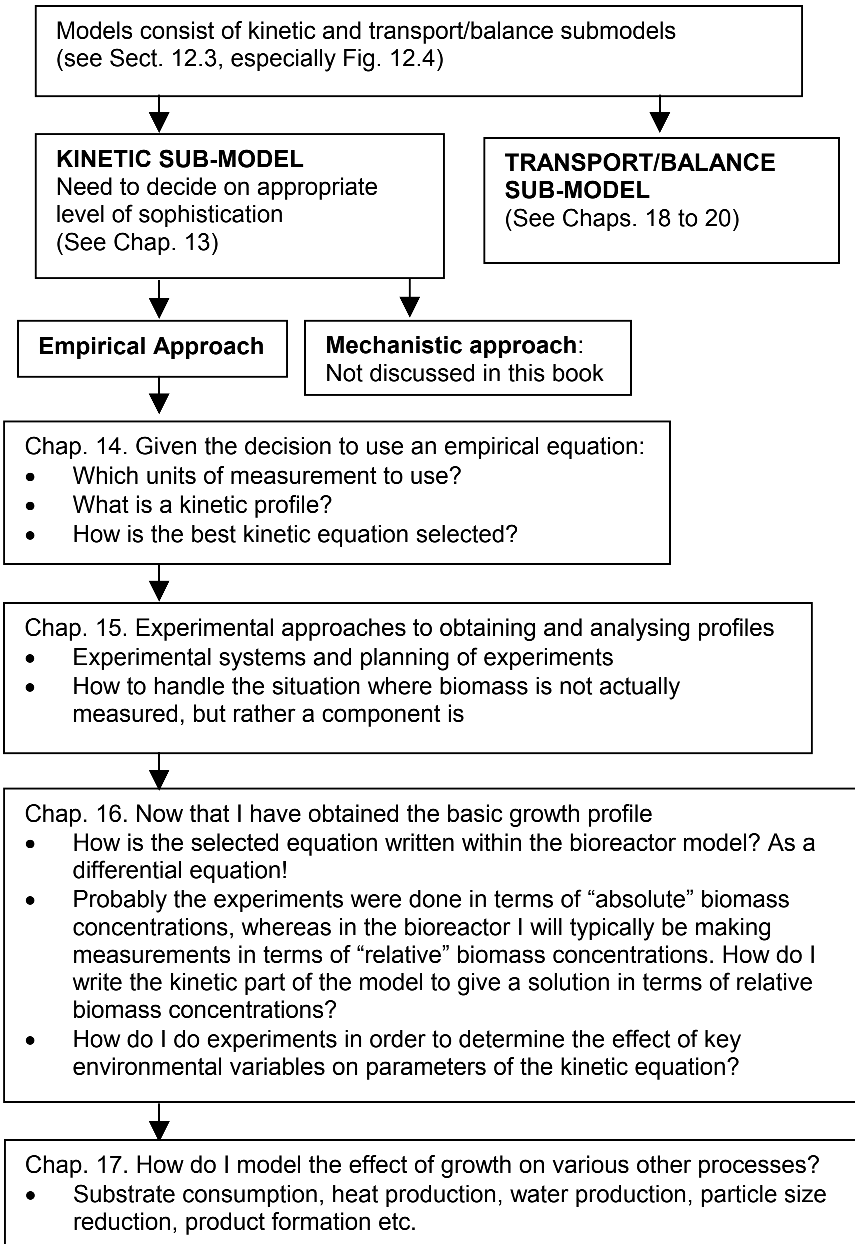


Fig. 14.1. An overview of how to go about establishing the kinetic sub-model of a mathematical model of an SSF bioreactor, showing how these issues are covered within various chapters of this book

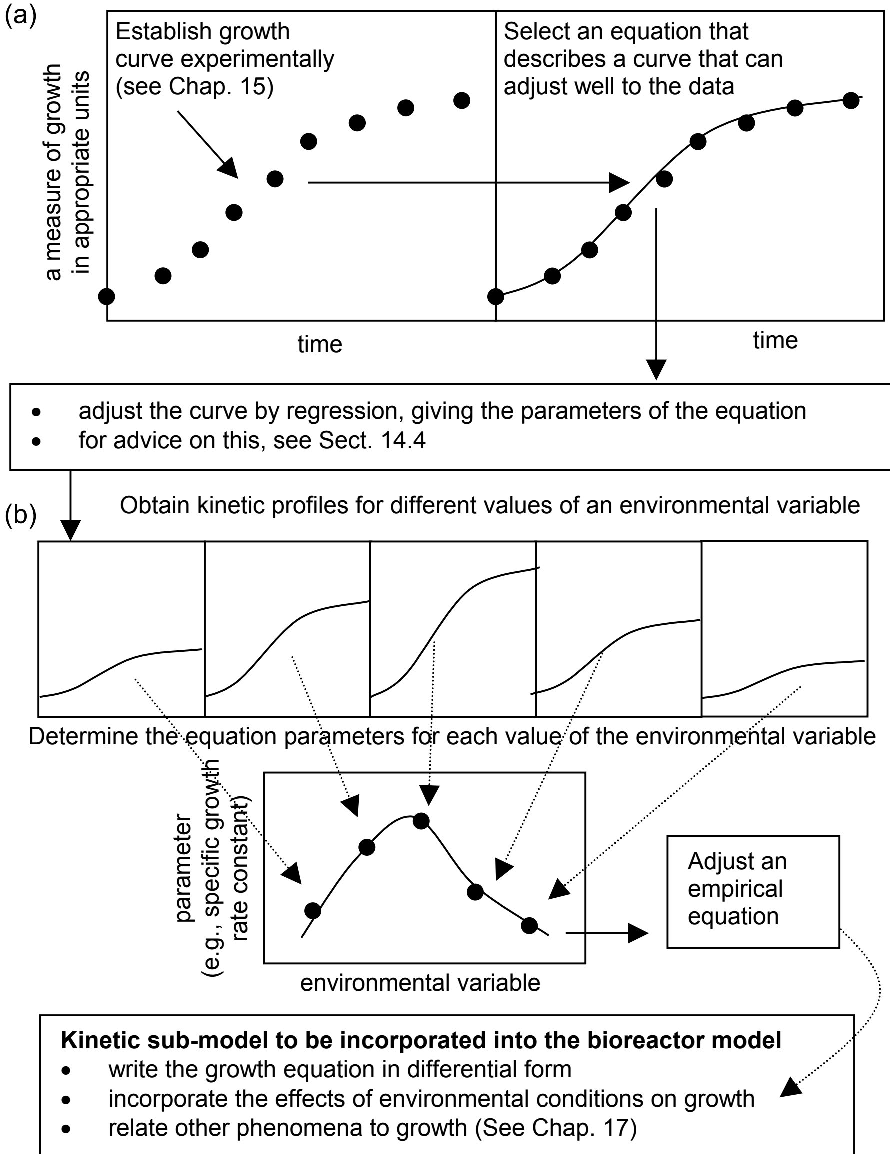


Fig. 14.2. The traditional approach to establishing the growth-kinetic equation. **(a)** A growth curve is established under optimal conditions and an empirical kinetic equation is selected that describes the curve well. **(b)** The parameters of the equation are expressed in terms of key environmental conditions (e.g., temperature) by repeating the growth curve experiment in different conditions, determining the growth parameters for each curve, and expressing the parameters as empirical functions of the environmental variable

14.2 How Will Growth Be Measured Experimentally?

14.2.1. The Problem of Measuring Biomass in SSF

The first experimental step in developing a kinetic model is to undertake a fermentation and plot the biomass content of the fermenting solid substrate against time (Fig. 14.2(a)). However, this immediately raises an experimental difficulty that is not faced in typical SLF processes (Fig. 14.3). In order to measure the dry weight of biomass directly, it is necessary to separate the biomass from solids. Many SSF processes involve filamentous fungi and, due to the penetration of the mycelium into the solid substrate, it is often impossible to remove the biomass quantitatively from the substrate, meaning that indirect methods of biomass measurement have to be used. Even in fermentations that involve unicellular organisms, although it may be possible to suspend many of the cells that are adhered to the particle surface and let the solid material sediment, the measurements are likely to be inaccurate (see Sect. 14.2.2).

The difficulty in measuring biomass dry weight in SSF raises the question of whether it is really necessary to use the dry weight of biomass as an indicator of growth. In fact, in SLF it is usually so simple to measure the dry weight of biomass (Fig. 14.3) that thought is often not given to whether this is the best parameter. So why do we need to measure the dry weight of biomass? Our aim in writing the kinetic sub-model of the bioreactor model is to write an equation that describes changes in a key variable to which we can relate other key processes that have important effects on bioreactor performance, such as metabolic heat production and O_2 consumption. However, does this variable have to be the dry weight of biomass? Are heat production and O_2 consumption actually related to the amount of dry biomass in the system? Or are they related to the amount of actively metabolizing biomass in the system? Given that we are typically limited to indirect measurements of growth in SSF, is it really necessary to convert the indirect measurement into dry weight? The answer is that no, it is not essential to write the kinetic sub-model in terms of the dry weight of biomass; we can use any growth-related parameter to which the important growth-related processes can be linked. For example, it may be possible to couple all the important growth-related activities to experimentally determined respiration kinetics.

Having said this, it is important to note that many of the current bioreactor models do in fact base their kinetic sub-models on changes in the dry weight of biomass. Therefore this book recognizes that indirect measures of growth will typically be converted into estimates of the dry biomass. The point is that the approach presented in this book is not the only possibility; other approaches to modeling the kinetics are possible. These other approaches will follow the general principles that we develop here in terms of dry biomass measurements.

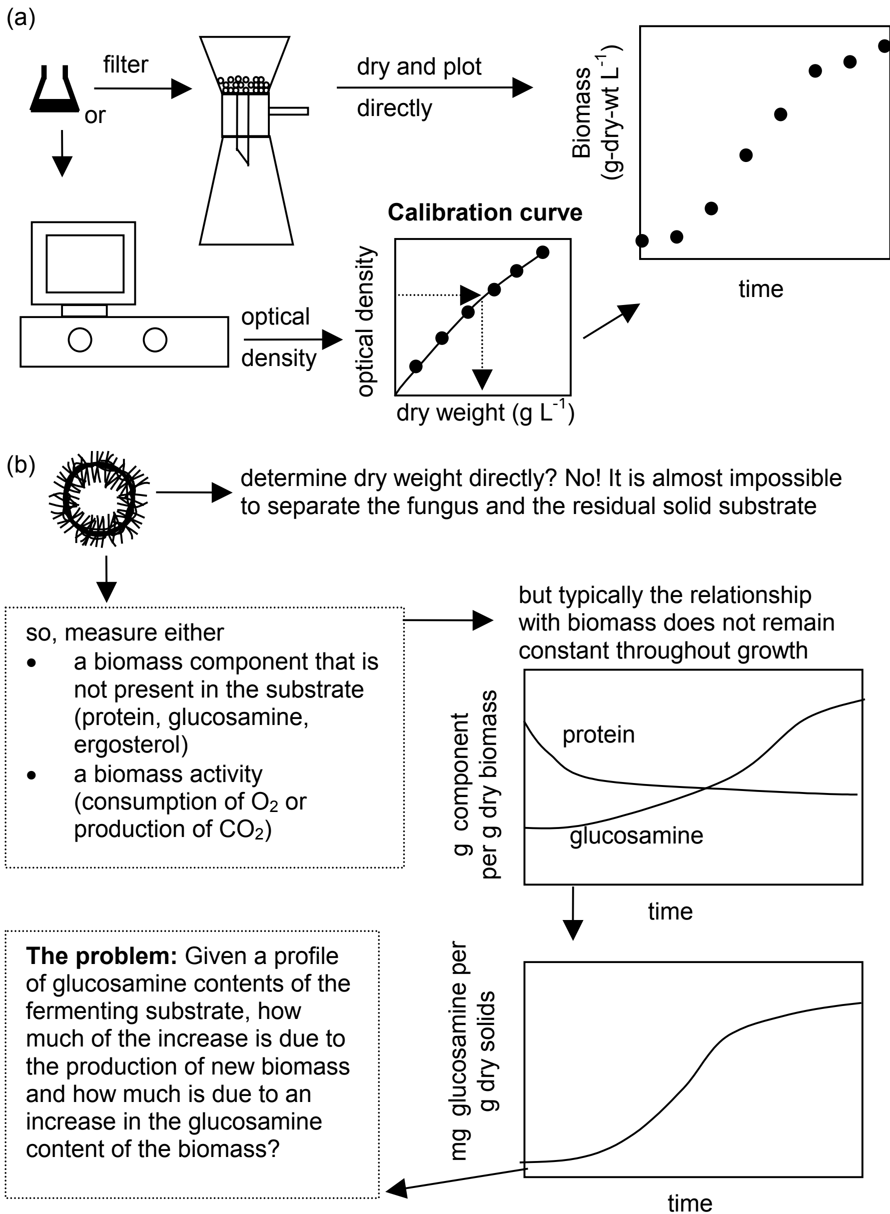


Fig. 14.3. A comparison of the ease of establishing biomass dry weight profiles. **(a)** In submerged liquid fermentation. **(b)** In solid-state fermentation

14.2.2 Indirect Approaches to Monitoring Growth

This section briefly mentions some of the direct approaches and various indirect approaches that can be used for monitoring growth in SSF systems. It is not intended to be an exhaustive review and it does not give protocols for the various methods. These should be searched for in original references. Some useful sources are given in the further reading section at the end of this chapter.

In some cases direct separation of the biomass is possible. With unicellular organisms it may be possible to dislodge the cells from the solid particles during a homogenization step and then to separate the solids from the suspended cells by sedimentation. However, some cells will remain adhered to the sedimented solids while some fine solid particles ("fines") liberated from the solids will not sediment. These fines will cause problems for determination of dry weight by filtration of the supernatant, since they will be erroneously counted as dry biomass. If viable count measurements are done on the supernatant, it is quite probable that the fines will have various cells adsorbed onto them, and these will give rise to only one colony per particle instead of one colony per cell.

In fungal fermentations, it is sometimes possible to digest the solid substrate within an aqueous enzyme solution, thereby allowing the mycelial biomass to be recovered by filtration. For example, this may be possible if the solid substrate is based on starch and contains little fiber, in which case the substrate can be hydrolyzed with amylases. However, some of the dry weight of biomass may be lost in this procedure and some solid residues may remain in the filtered biomass fraction. The efficiency of the recovery could be checked by submitting known masses of fungal mycelium, for example, from membrane filter culture (Chap. 15.3.1), to the hydrolysis and recovery procedure.

Various indirect methods rely on measurement of biomass components such as:

- **Ergosterol.** This is the predominant sterol in the cell membrane of many fungi, and is typically not found in plant material. It can be quantitatively measured by gas chromatography, HPLC, or UV spectrometry.
- **Glucosamine.** This is produced by the hydrolysis of chitin, which many fungi contain in their cell wall. It is typically not found in materials of plant origin. The hydrolysis of the biomass and subsequent determination of glucosamine by the chemical method can be quite tedious. It may be preferable to determine the glucosamine in the hydrolysate by HPLC.
- **Protein.** Protein is a major cell component. However, it is present in many plant materials and, if present, it will be impossible to know the proportion of protein in the sample that comes from the substrate, and the proportion that comes from the biomass, since the microorganism will typically hydrolyze the protein during growth. Therefore use of protein determination as an indicator of growth is restricted to cases in which the substrate contains negligible protein.

Unfortunately, the content of all of these components within the biomass can vary with culture conditions and with the age of the fungal mycelium. This greatly complicates the conversion of indirect measurements into estimates of the dry weight of biomass.

Other indirect methods rely on detecting activities of the biomass. Of these, the consumption of O_2 and production of CO_2 are most important. Gas metabolism is potentially a very important growth activity, especially since the rate of heat evolution will typically be directly proportional to the O_2 consumption rate, at least for an aerobic process. Further, the overall O_2 consumption within a bioreactor can be used for on-line monitoring of the growth process, even though it is not necessarily a simple matter to convert the O_2 consumption profile into a trustworthy biomass growth profile. Due to the importance of O_2 uptake measurements, the experimental use of this method in growth kinetic studies is discussed in Chap. 15.

The above discussion shows that several questions must be answered when selecting an appropriate indirect method for estimating growth:

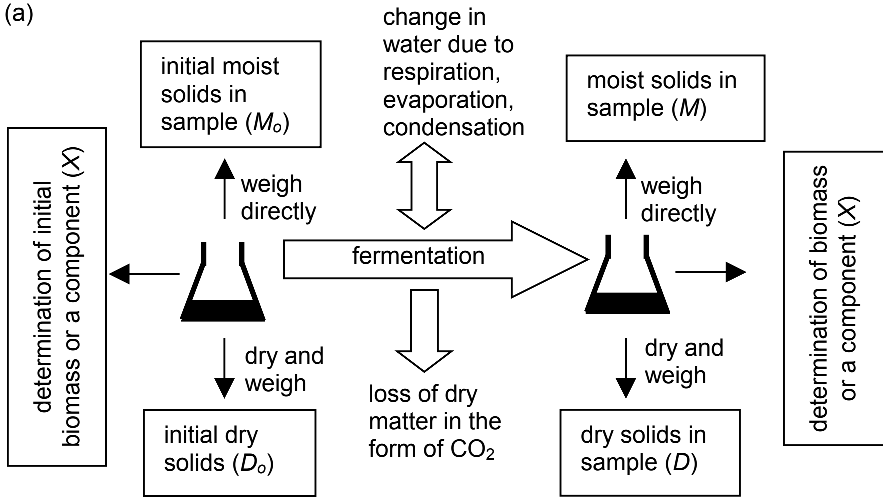
- Is the component that is to be measured also present in the substrate?
- What time and resources are required for processing of the samples?
- To what degree does the relationship between the activity or component and the amount of biomass present change during the fermentation?

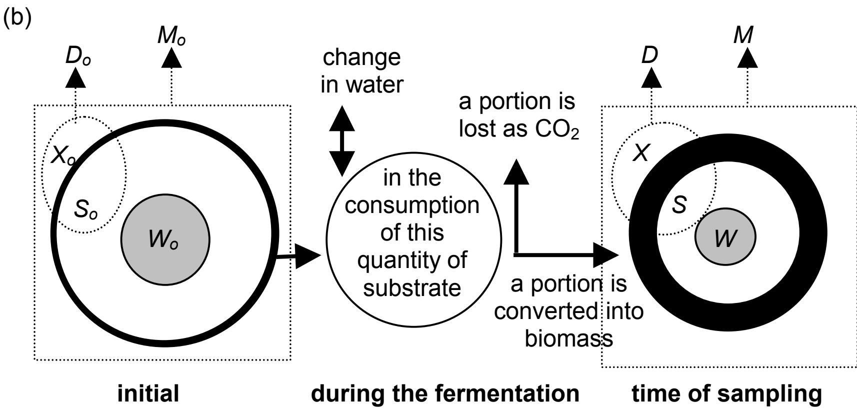
It may or may not be desired to convert an indirect measurement into an estimation of the biomass itself. If it is desired to do so, then the measurement method must be calibrated. In other words, the organism must be grown in a system that allows the dry weight of biomass to be measured in addition to the component or activity. These issues are discussed in Chap. 15.3.

14.3 What Units Should Be Used for the Biomass?

Once a direct or indirect measurement method has been selected, it will be used to give an estimate of the amount of biomass in samples removed over the course of the fermentation, allowing the construction of a kinetic profile. However, there remains a question: “What units will be used to express the biomass concentration in the kinetic profile?” The importance of this question becomes apparent when it is realized that various different units have been used to construct kinetic profiles in the past. These various methods are compared in Fig. 14.4, which also indicates the meaning of the various symbols used below:

- **grams of biomass or component per gram fresh sample.** In this case the sample is removed and weighed directly, the amount of biomass or component then being measured and divided by the fresh weight of the sample (i.e., X/M);
- **grams of biomass or component per gram dry sample.** In this case the sample is removed, dried in an oven at around 50-70°C and then weighed, after which the biomass or component is measured and divided by the dry weight of the sample (i.e., X/D). Note that if the analytical method used would be adversely affected by a drying step, the sample can be divided, with the water content being determined by drying of one fresh sub-sample and the other fresh sub-sample being used for biomass determination.





☐ = substrate ■ = biomass ▒ = water

$$C_{XM} = \frac{\text{biomass}}{\text{fresh matter at time of sampling}} = \frac{X}{X+S+W} \qquad C_{XR} = \frac{\text{biomass}}{\text{dry matter at time of sampling}} = \frac{X}{X+S}$$

$$C_{XW} = \frac{\text{biomass}}{\text{initial fresh matter}} = \frac{X}{X_o+S_o+W_o} \qquad C_{XA} = \frac{\text{biomass}}{\text{initial dry matter}} = \frac{X}{X_o+S_o}$$

Fig. 14.4. Various manners in which the biomass content can be expressed. **(a)** The various measurements that can be made. **(b)** The biomass content will be calculated as a different number depending on what is included in the denominator

- **grams of biomass or component per gram initial fresh sample.** In this case the sample is removed and the amount of biomass or component is determined. To calculate the biomass content, the amount of biomass is divided not by the mass of fresh solids in the sample, but by the mass of fresh solids present at the time of inoculation (i.e., X/M_o);
- **grams of biomass or component per gram initial dry substrate.** In this case the sample is removed and the amount of biomass or component is determined. To calculate the biomass content, the amount of biomass is divided not by the mass of dry solids in the sample, but by the mass of dry solids present at the time of inoculation (i.e., X/D_o).

Of course, if sufficient data is available about how the water content and total dry solids vary during the fermentation, it is possible to calculate the biomass concentration in any of the above units. It is easy to obtain sufficient data to do this in laboratory experiments, but not so easy within a bioreactor.

So which is the most appropriate set of units to use in analyzing kinetics? This question will be addressed in Sect. 14.3.5 after considering the consequences of using each set of units.

14.3.1 Grams of Biomass per Gram of Fresh Sample

Expressing the biomass concentration per gram of fresh sample (C_{XM}) means that the denominator depends on changes in three factors, the mass of biomass (X), the mass of residual dry substrate (S), and the mass of water (W):

$$C_{XM} = \frac{X}{X+S+W} = \frac{X}{D+W} = \frac{X}{M}. \tag{14.1}$$

The sum of X and S is the total mass of dry solids (D). The sum of the dry solids and the water gives the total mass of the moist solids (M).

A biomass content expressed in these terms will not only be influenced by the consumption of dry matter, but will also be influenced by changes in the water content of the substrate, these changes arising from metabolic water production and evaporation. At the extreme, even if the organism is neither growing nor consuming substrate, C_{XM} can increase due to evaporation of water from the substrate.

14.3.2 Grams of Biomass per Gram of Dry Sample

Expressing the biomass in terms of the amount of dry sample removes the effect of changes in the water content on the apparent biomass concentration. However, due to the conversion of solid organic matter into CO_2 during the fermentation, the amount of solid material in the bioreactor can change significantly during the fermentation. In this case, the change in the biomass content expressed on the basis of “g of biomass per g of dry sample” arises from two sources: increase in the mass of biomass and decrease in the mass of solids. It is possible to have a situa-

tion where the microorganism is not growing, but is metabolizing to maintain itself. In such a situation the biomass concentration expressed per mass of dry sample will increase due to the loss of dry matter as CO_2, despite the fact that the biomass is not increasing.

The symbol C_{XR} (g-dry-biomass g-dry-solids^{-1}) can be used to represent a biomass content of this kind. It is given by:

$$C_{XR} = \frac{X}{X+S} = \frac{X}{D}. \tag{14.2}$$

14.3.3 Grams of Biomass per Gram of Initial Fresh or Dry Sample

The effect of water and dry matter loss on the apparent biomass concentration can be removed by expressing the biomass on the basis of an initial quantity of solids. One possibility would be to define the biomass concentration in terms of the initial mass of moist solids:

$$C_{XW} = \frac{X}{X_o + S_o + W_o} = \frac{X}{D_o + W_o} = \frac{X}{M_o}, \tag{14.3}$$

where the subscript "*o*" indicates initial masses of the various components. However, this has been used only rarely, since it is more common to work in terms of dry solids:

$$C_{XA} = \frac{X}{X_o + S_o} = \frac{X}{D_o}. \tag{14.4}$$

C_{XA} has the units of g-biomass g-initial-dry-solids^{-1}, these units typically being written as g-biomass g-IDS^{-1}.

Unlike the other methods of expressing biomass concentration, these measures will only change in response to changes in the amount of biomass. In the absence of growth, they will not change as a result of changes in either moisture content or dry solids content. Therefore they will be referred to as "absolute biomass concentrations". Concentrations expressed in the manner shown in Eqs. (14.1) and (14.2) will be referred to as "relative biomass concentrations".

There are other absolute measures of biomass:

- the amount of biomass per gram of inert support material, which can be used in some cases where an inert support matrix is impregnated with nutrients;
- the absolute amount of biomass within the bioreactor;
- the amount of biomass per unit volume of the substrate bed. Note that this is only an absolute biomass concentration in those cases in which the bed volume does not change significantly during the process. Biomass per unit volume is typically not used to express biomass concentration in laboratory-scale experiments, but biomass concentrations may be expressed in this manner within mathematical models of bioreactors.

In this book we will use the symbol *X* to represent either the absolute mass of biomass in the bioreactor or the mass of biomass per m^3 of substrate bed. Other symbols will be used to represent a concentration based on a denominator that does not change, such as grams initial dry solid, or grams of inert support material.

14.3.4 Which Set of Units Is Best to Use for Expressing the Biomass?

It is probably best, in the kinetic studies undertaken in the laboratory, to express the biomass concentration on an absolute basis. This is because key phenomena that will be included in the bioreactor model (such as the production of waste metabolic heat, the consumption of O_2, and the production of CO_2) depend directly on the absolute amount of biomass.

However, as will become obvious in the following section, absolute biomass contents have not always been used in growth profiles reported in the literature (Viccini et al. 2001). This must be kept in mind when analyzing kinetic profiles taken from the literature. In any case, it is not difficult to convert between absolute and relative concentrations if the yield and maintenance coefficients are known. A method of doing this is presented in Chap. 16.2.

14.4 Kinetic Profiles and Appropriate Equations

This section summarizes the various shapes of kinetic profiles that have observed in the literature, the empirical equations that have been used to describe them, and the manner in which the parameters of the equations are estimated.

Four differently shaped kinetic profiles have been reported in various SSF systems: "linear", "exponential", "logistic", and "deceleration". The general shapes of these kinetic profiles are shown in Fig. 14.5 (Viccini et al. 2001).

The equations that describe these curves are shown in Table 14.1. The task is to select the curve that is best able to fit the particular experimental results for biomass, or some indicator of biomass. Note that other shapes of growth curves are possible, in which case it is necessary to propose a new equation that describes the shape of the new curve. Curve selection and fitting will typically be done by regression. In regression analysis the model parameters are adjusted until the sum of squares of deviations between the experimental results and the corresponding values on the fitted curve are at a minimum (Fig. 14.6). There are many software packages that can be used to do regression. After doing the regression for each of the different equations, the curve chosen will typically be the one for which the sum of squares of deviations is the smallest. However, there may also be reasons for preferring a specific equation, even if it does not give the best fit to the data. For example, the logistic equation is usually preferred because often it is possible to use it to describe the whole growth curve adequately, whereas with the other kinetic equations the growth cycle needs to be broken up into intervals, each with a different equation. The regression analysis also gives the values for the

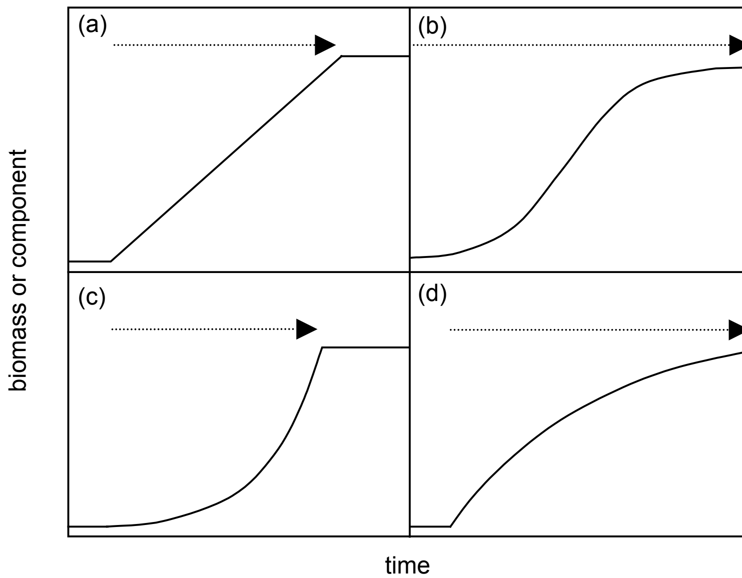


Fig. 14.5. Various types of kinetic profiles that have been found in SSF. The arrows indicate the parts of the profile that correspond to the kinetic type. **(a)** linear; **(b)** logistic; **(c)** exponential; **(d)** deceleration

parameters of the kinetic equation, at least for the conditions under which the experiment was done. Note that, as will be discussed in Chap. 16, the parameters will typically not appear in the final kinetic equation as constants, but rather as functions of key environmental variables.

The logistic equation fits reasonably well to around 75% of the literature profiles obtained in SSF systems (Viccini et al. 2001). The other 25% of profiles are described acceptably by one of the other three equations. Note that many of the experimental growth profiles obtained in the past were not done with kinetic analysis in mind. As a consequence, often there are relatively few data points during the period of rapid growth. This can lead to a situation in which several of the equations can adjust reasonably to the data, it not being possible to determine which gives the best fit. Chapter 15 gives some advice about how to plan experiments to avoid such problems.

Other important issues related to the kinetic analysis that you would need to do for your own system are presented in the following paragraphs.

Use absolute concentrations. As noted in Sect. 14.3.4, it is advisable to undertake the experiments in such a manner as to be able to plot the data in terms of absolute concentrations and to fit an equation to this absolute concentration data.

Table 14.1. Equations that have been used to describe growth profiles or parts of growth profiles in SSF systems[a]

Name	Equation	Equation number	Parameters to be found by regression
Linear	$C = C_o + kt$	(14.4)	C_o, k
Exponential	$C = C_o e^{-\mu t}$	(14.5)	C_o, μ
Logistic	$C = \dfrac{C_m}{1 + \left(\dfrac{C_m}{C_o} - 1\right) e^{-\mu t}}$	(14.6)	C_o, C_m, μ
Deceleration	$C = C_o \exp\left(A(1 - e^{-kt})\right)$	(14.7)	C_o, A, k

[a] In the past these equations have been used for biomass concentrations expressed on both absolute and relative bases.

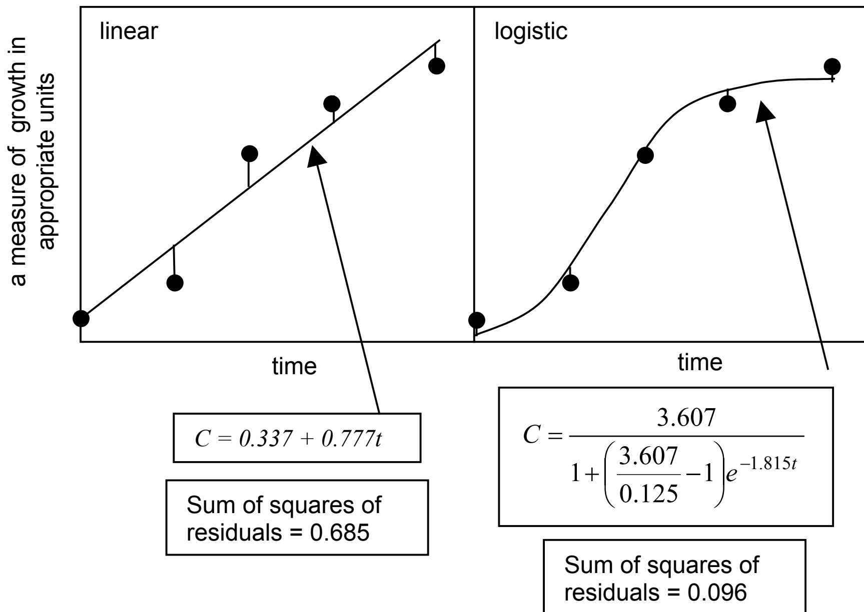


Fig. 14.6. How regression analysis is used to determine the most appropriate kinetic equation and the values of the parameter of this equation that give the best fit to the experimental data. In this case the logistic equation would be selected since it gives a better fit, as indicated by the smaller sum of squares of residuals. The residuals are the vertical lines that represent, for a particular time, the difference between the experimental value and the value predicted by the equation

Take care to select the appropriate interval for the regression analysis. The kinetic equations in Table 14.1 may apply to only part of the overall kinetic profile. There might be lag and stationary phases not described by these equations, in which case it is necessary to select carefully the region of the growth profile to which the kinetic equation will be fitted. For example:

- None of these equations explicitly describe a lag phase. However, the exponential and logistic equations may give apparent lag phases on a linear-linear plot if the initial biomass concentration is very low.
- The exponential and linear equations do not describe any limitation on growth. Of course if the growth curve is followed for long enough, the biomass profile must eventually show a maximum concentration (C_m). For these equations it may be appropriate to define a separate stationary phase. The logistic and deceleration equations can describe a stationary phase, which occurs at C_m for the logistic equation and at $C_o.e^A$ for the deceleration equation. These equations make no assumptions about the mechanism of limitation. In different systems limitations on the maximum amount of growth might be related to the exhaustion of essential nutrients, to the accumulation of inhibitory end products of metabolism, or to steric considerations (i.e., through the biomass "filling" the physical space available, noting that, even at their maximum packing density, fungal hyphae occupy only about 34% of the available volume (Auria et al. 1995)). Therefore the significance of C_m may vary from system to system. Typically it will be treated as a simple empirical parameter.
- There may even be a decline or death phase, which is not described by any of these equations. The modeling of death kinetics is discussed in Chap. 16.4.

Keep the environmental conditions constant. The parameters of the equation will change for cultures grown in different conditions, for example, at different temperatures, on different substrates, or with different O_2 concentrations in the gas phase. Therefore, during the fermentation the conditions should be held as constant as possible. This may not be simple, even at small scale. Difficulties in maintaining constant conditions and experimental strategies to minimize deviations are discussed in Chap. 15. Note that in more sophisticated studies, in which the effects of varying conditions on growth are investigated, it may actually be desirable to vary the conditions in a deliberate manner during the fermentation.

14.5 Conclusions

So far we have addressed the graphical and mathematical issues associated with constructing and analyzing the kinetic profile. The next chapter gives advice about the experimental techniques that may need to be used.

Further Reading

A survey of kinetic profiles in SSF systems

Viccini G, Mitchell DA, Boit SD, Gern JC, da Rosa AS, Costa RM, Dalsenter FDH, von Meien OF, Krieger N (2001) Analysis of growth kinetic profiles in solid-state fermentation. Food Technol Biotechnol 39:271–294

Use of response surface analysis for determining the optimum medium and conditions for growth and product formation

Kalil SJ, Maugeri F, Rodrigues MI (2000) Response surface analysis and simulation as a tool for bioprocess design and optimization. Process Biochem 35:539–550

Lekha PK, Chand N, Lonsane BK (1994) Computerized study of interactions among factors and their optimization through response surface methodology for the production of tannin acyl hydrolase by *Aspergillus niger* PKL 104 under solid state fermentation. Bioprocess Eng 31:7–15

General reviews of methods for determination of biomass in SSF

Desgranges C, Vergoignan C, Georges M, Durand A (1991) Biomass estimation in solid state fermentation I. Manual biochemical methods. Appl Microbiol Biotechnol 35:200–205

Desgranges C, Georges M, Vergoignan C, Durand A (1991) Biomass estimation in solid state fermentation II. On-line measurements. Appl Microbiol Biotechnol 35:206–209

Matcham SE, Wood DA, Jordan BR (1984) The measurement of fungal growth in solid substrates. Appl Biochem Biotechnol 9:387–388

Mitchell DA (1992) Biomass determination in solid-state cultivation In: Doelle HW, Mitchell DA, Rolz CE (eds) Solid Substrate Cultivation. Elsevier Applied Science, London, pp 53–63

Specific methods for determination of biomass in SSF

Acuna G, Giral R, Thibault J (1998) A neural network estimator for total biomass of filamentous fungi growing on two dimensional solid substrate. Biotechnol Techniques 12:515–519

Cordova-Lopez J, Gutierrez-Rojas M, Huerta S, Saucedo-Castaneda G, Favela-Torres E (1996) Biomass estimation of *Aspergillus niger* growing on real and model supports in solid state fermentation. Biotechnol Techniques 10:1–6

Davey CL, Penaloza W, Kell DB, Hedger JN (1991) Real-time monitoring of the accretion of *Rhizopus oligosporus* biomass during the solid-substrate tempe fermentation. World J Microbiol Biotechnol 7:248–259

Dubey AK, Suresh C, Umesh Kumar S, Karanth NG (1998) An enzyme-linked immunosorbent assay for the estimation of fungal biomass during solid-state fermentation. Appl Microbiol Biotechnol 50:299–302

Ebner A, Solar I, Acuna G, Perez-Correa R, Agosin E (1997) Fungal biomass estimation in batch solid substrate cultivation using asymptotic observation. In: Wise DL (ed), Global Environmental Biotechnology, Kluwer Academic Publishers, Dordrecht, pp 211–219

Matcham SE, Jordan BR, Wood DA (1985) Estimation of fungal biomass in a solid substrate by three independent methods. Appl Microbiol Biotechnol 21:108–112

Ooijkaas LP, Tramper J, Buitelaar RM (1998) Biomass estimation of *Coniothyrium minitans* in solid-state fermentation. Enzyme Microbial Technol 22:480–486
Penaloza W, Davey CL, Hedger JN, Kell DB (1992) Physiological studies on the solid-state quinoa tempe fermentation, using on-line measurements of fungal biomass production. J Sci Food Agr 59:227–235
Ramana Murthy MV, Thakur MS, Karanth NG (1993) Monitoring of biomass in solid state fermentation using light reflectance. Biosensor Bioelectronics 8:59–63
Roche N, Venague A, Desgranges C, Durand A (1993) Use of chitin measurement to estimate fungal biomass in solid state fermentation. Biotechnol Adv 11:677–683
Rodriguez Leon JA, Sastre L, Echevarria J, Delgado G, Bechstedt W (1988) A mathematical approach for the estimation of biomass production rate in solid state fermentation. Acta Biotechnol 8:307–310
Terebiznik MR, Pilosof AMR (1999) Biomass estimation in solid state fermentation by modeling dry matter weight loss. Biotechnol Techniques 13:215–219
Weber FJ, Tramper J, Rinzema A (1999) Quantitative recovery of fungal biomass grown on solid kappa-carrageenan media. Biotechnol Techniques 13:55–58
Wiegant WM (1991) A simple method to estimate the biomass of thermophilic fungi in composts. Biotechnol Techniques 5:421–426
Wissler MD, Tengerdy RP, Murphy VG (1983) Biomass measurement in solid-state fermentations using ^{15}N mass spectrometry. Dev Ind Microbiol 24:527–538
Wood DA (1979) A method for estimating biomass of *Agaricus bisporus* in a solid substrate, composted wheat straw. Biotechnol Lett 1:255–260