

## Chapter 2

# General Results

**Abstract** This chapter is the most general of this book. It does not deal with biotechnology or microbiology in particular. However, it is necessary for these two disciplines which make up the core of this book. In fact, this chapter attempts to find the general theoretical bases of the original approach of polyphasic dispersed systems (PDS). It critically reconsiders the most common assumptions directly used in physical chemistry systems and, in particular, the usual ways of theoretical representation, such as mathematical modeling, for example. Based on the concept of description level described in the previous chapter, it analyzes key hypotheses such as the homogeneity of a system. It attempts, maybe sometimes in a too rudimentary way, to define new concepts, such as the minimum volume of a system required to implement the original concepts of the PDS; it formally introduces the different types of concentrations relevant to a correct description of a phenomenon and the consequences of these definitions on the assessment of experimental measurements. This chapter also provides the basis for calculating the mass balance in a polyphasic dispersed system and shows how this approach can bring to light mass fluxes that are cryptic in the classic modes of descriptions. Finally, last but not least, it draws the attention to the possibility, regarding doubly open systems (in a thermodynamic sense), to envisage a “physicochemical relativity” in writing the mass balances.

### 2.1 Definition of a Polyphasic Dispersed System (PDS)

Polyphasic dispersed systems such as we consider, differ markedly from the dispersed systems of the physicist. It is principally a two-phase system that belongs to this category, such as aerosols, mousses, emulsions, sols and gels, etc. The dispersed particles are particles of between  $10^{-3}$  and  $1\text{ }\mu\text{m}$  and are made up of micelles, bilayers, and bilayer vesicles (of the order of  $5\text{--}10\text{ nm}$ ) (Takeo 1999). In general, these systems display a certain amount of stability in the sense that they can support themselves, over a greater or shorter timescale, even if they are a closed

system. This property is due to Brownian movement or repulsive forces (electrostatic, for example) according to the size of the particles. They can be the site of transformation processes (aggregation, flocculation, etc.) that can give rise to the separation of phases, but what distinguishes them most, perhaps, from the PDS such as we imagine them, are the dispersion forces. In the cases considered above, these forces are generated by the system itself (essentially repulsive forces; refer to Takeo 1999) and can be considered as internal. In the PDS, on the other hand the dispersive forces are mainly external (such as mechanical agitation, for example). The second fundamental difference concerns the size of the dispersed particles. The scale of the dimensions is between micrometer and millimeter (even centimeter). In spite of major differences, there are obviously things in common that will not be developed here. (These are concepts that have already been brought to mind such as certain repulsive forces (or attraction forces) or even thermodynamic considerations, etc.). So we can almost consider that the class of systems that will be taken into account below is independent of the dispersed systems as presented at the beginning of the section. However, it was important to make clear the distinction to avoid misunderstandings.

A polyphasic dispersed system will be defined as a system composed of several phases (solid, liquid, gaseous) that are closely distributed one in another and for the most part generally maintained in this dispersed state by external dispersion forces such as mechanical agitation. The PDS are thus intrinsically unstable systems. The interface between whatever two phases is therefore discontinuous and limited by phase fragments that we will designate as micelles (to avoid either creating a neologism or having to use periphrases).

Very generally, Fig. 2.1 shows a PDS of  $n$  phases  $(\phi_1, \phi_2, \phi_3, \dots, \phi_n, \dots)$ .

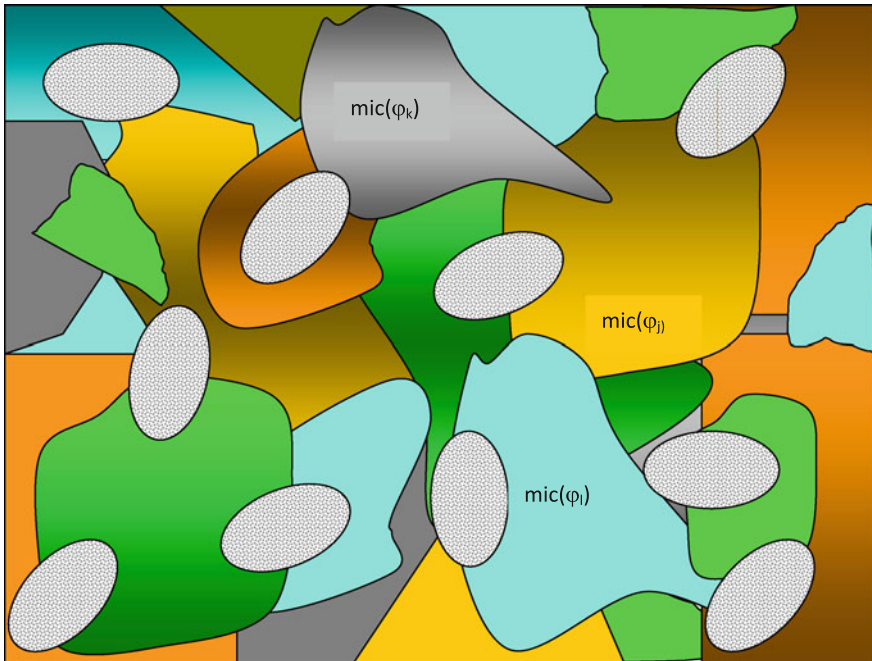
This PDS is called “compact” because the micelles  $(m(\phi_i))$  form a precise division of the system and it is not possible to travel through a whole phase without crossing at least one interface.

Figure 2.2 shows a dilute PDS where there is a path (C) that makes it possible to travel through the whole phase  $\phi_1$  without crossing a single interface. In this case, phase  $\phi_1$  is called the “dispersing phase” and it does not form micelles.

There is an intermediary case between the compact and the dilute system; this is called the dense system where dispersing phase micelle can be found (a dispersing phase micelle is a fragment from dispersing phase that is isolated from the phase by micelles from other phases). A phase from a PDS can also be defined as a whole of micelles of the same kind, whether these are in a closed system or an open system and if the input/output fluxes include micelles. (This concept of addition of input/output fluxes will become clearer later in the text.)

In the most general case, there can exist several types of different micelles in one and the same phase. So, for example, the solid phase can be made up of several types of different solid micelles. The liquid phase can also be made up of different liquid micelles formed of non-miscible liquids. (An example in a gaseous phase is not easy to bring to mind.)

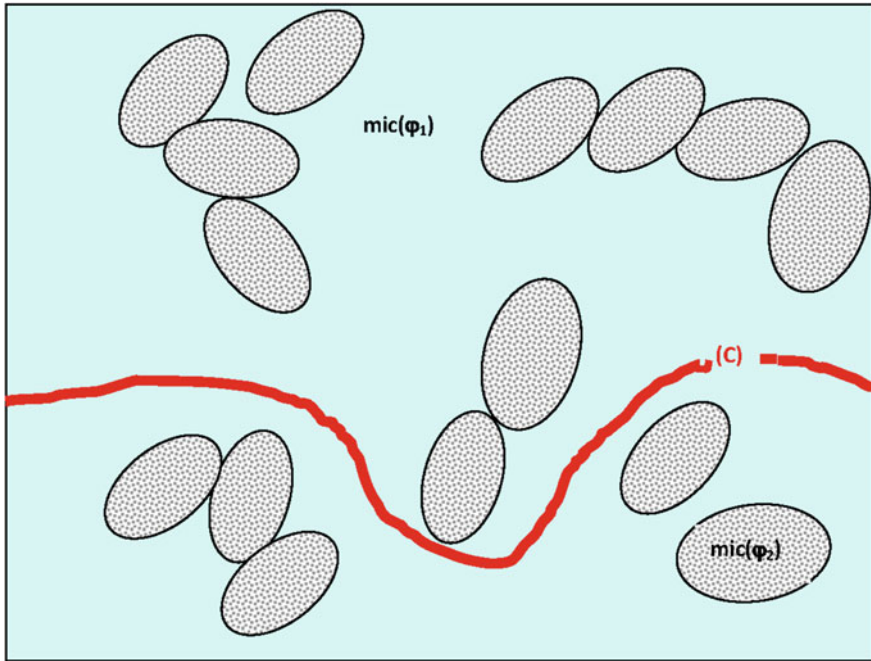
Generally, the number of micelles in a PDS (closed or open) is not constant. For example, the number of air bubbles can diminish by coalescence; the number of



**Fig. 2.1** Compact Polyphasic System. A compact PDS gives rise to a perfect division of the system and there is no path along which to travel through an entire phase without crossing at least one interface ( $\text{mic}(\varphi_i)$  should be read as “micelle belonging to phase  $i$ ”)

solid micelles can diminish by aggregation or increase by fragmentation; two types of micelles can combine to form a new type of micelle; in an open system, inflows and/or outflows of micelles can exist, etc. Nevertheless, if there is a steady state, this is characterized by a number of constant miracles. The steady state can apply to the whole of the micelles in the system or just to certain classes of micelles. By way of an example, an aerated culture can be composed of a constant number of solid micelles (cells, for example), whereas the quantity of gaseous micelles (bubbles) can vary over time.

However, the preceding considerations are not enough to define completely the PDS. In the way it has been described until now, the PDS appears clearly as a heterogeneous system. To complete the definition, the notion of pseudohomogeneity must be introduced. This means that in spite of the intrinsic heterogeneous character of the system, there is a certain level of description in which the element of the volume of the system can be considered as homogeneous (from a functional point of view). The element of volume in question is necessarily much bigger than the mean volume of one micelle but much less than the volume of the entire system. If this notion of pseudohomogeneity does not apply, the whole of the micelles and phases, etc., that are described above would only constitute a collection of objects that could not be treated as a continuous medium. Now this property is



**Fig. 2.2** Dilute Polyphasic System. In a dilute polyphasic system, there is a dispersing phase that features a path that makes it possible to travel through the entire phase without crossing an interface

indispensable to a certain independence regarding the spatial characteristics of the system and to the derivation (mathematically) of certain functions, etc. The option of choosing a level of description that makes it possible to treat the system as homogeneous, whereas in reality it is not (pseudohomogeneous) is the cornerstone of the definition of PDS. All mathematical theory and modeling that follow are based on this property of pseudohomogeneity. In reality, in the literature, the property of pseudohomogeneity is often implicit and considered as given. It follows that the relevance of representation or of modeling is not necessarily guaranteed.

## 2.2 Characteristic Volume and Pseudohomogeneity

The concept of pseudohomogeneity does not apply if a system can be divided into elements of volumes that are statistically equivalent from the point of view of their properties. These elements of volume must still be quite small in comparison with the total volume so that the medium, considered on a small scale, can appear as continuous. The same problem presents itself in physics when attempts are made to explain the macroscopic properties of a system (thermodynamics, for example)

beginning with properties that are on a microscopic level (atoms, molecules ...). In this case the dimensions of the particles are of the order of the angstrom ( $\text{\AA}$ ;  $10^{-10}$  m) and their number is of the order of Avogadro's number ( $6.023 \times 10^{23}$ ). On this scale, a macroscopic system of a solution of HCl or of a gas in an equilibrium of let us say  $1 \text{ cm}^3$  can be considered as perfectly homogeneous. It is the role of statistical physics to analyze the relationship between microscopic distributions and macroscopic properties, and this represents a considerable branch of physics. Obviously, the aim here is not to develop a sophisticated statistical theory, but simply to try to understand more quantitatively, the concept of pseudohomogeneity that is often accepted without saying.

If anyone has seen a suspension of baker's yeast, well stirred in a 250 ml conical flask, they will willingly concede that this beige, milky liquid of uniform color is homogenous. However, an elementary microscopic examination makes it appear as a heterogeneous system, formed of cell structures that are very different from the medium of the culture and are more or less regularly placed in the microscope's field. At this level of observation, there is no doubt that the system is heterogeneous while we are ready to consider some dozens of  $\text{cm}^3$  of this suspension as homogenous.

The concept of homogeneity of a system could be defined in the following manner: two (or more) elements of equal volume sampled randomly from two (or more) points of the system, present exactly the same properties (physical, chemical, etc.). It is already known that this type of homogeneity does not exist in reality and the term "exactly" should be replaced by "statistically." So the relevant question is from what size two elements of volume can have statistically the same properties. To come back to the example of the suspension of yeasts, it seems inevitable that it should be considered, in reality, as heterogeneous but if a large volume is considered, it might be conceded that it can appear homogenous. On a certain scale, it can be said that it is pseudohomogenous. However, this property can be used to study the system only if the element of volume from which the suspension can be called pseudohomogenous is small in comparison with the whole system.

A "naive" step to estimate the size of this element of volume can be attempted. Take into consideration that each micelle of the system can be characterized by a value of between 0 and 1. In the case of a cell, it can be a question of, for example, its size characterized by the relationship of its present size and its maximum size before division; or the relationship with a quantity of constitutive enzymes at maximum level that it can present; or the relationship with the present number of several receptors in the membrane in relationship to the maximum quantity, etc., a characteristic size  $\xi$  (a property) is defined as:

$$\xi = \frac{x}{\max(x)}; \quad 0 \leq \xi \leq 1 \quad (2.2.1)$$

Let us take the equally probable case of each micelle having the same probability of finding itself in state  $\xi_1$  as in state  $\xi_2$ . In other words, we consider that for  $\xi$  there is a uniform distribution. Over the interval  $[0, 1]$  the average value for  $\xi$  is  $\bar{\xi} = 0.5$  and the variance is  $\sigma_{\xi}^2 = 1/12$  (Kaufman 1965).

By numeric simulation, we have estimated the average and the variance of the property  $\zeta$  in two elements of volume that have a growing number of micelles. Estimation of the average has been calculated by

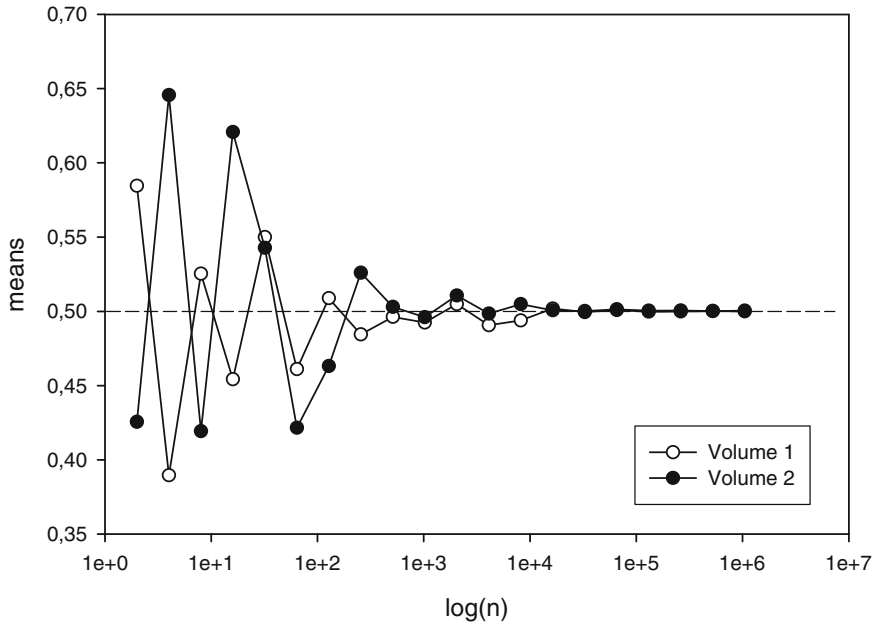
$$\bar{\zeta} = \frac{1}{n} \sum_{i=1}^n \zeta_i$$

and that of the variance by,

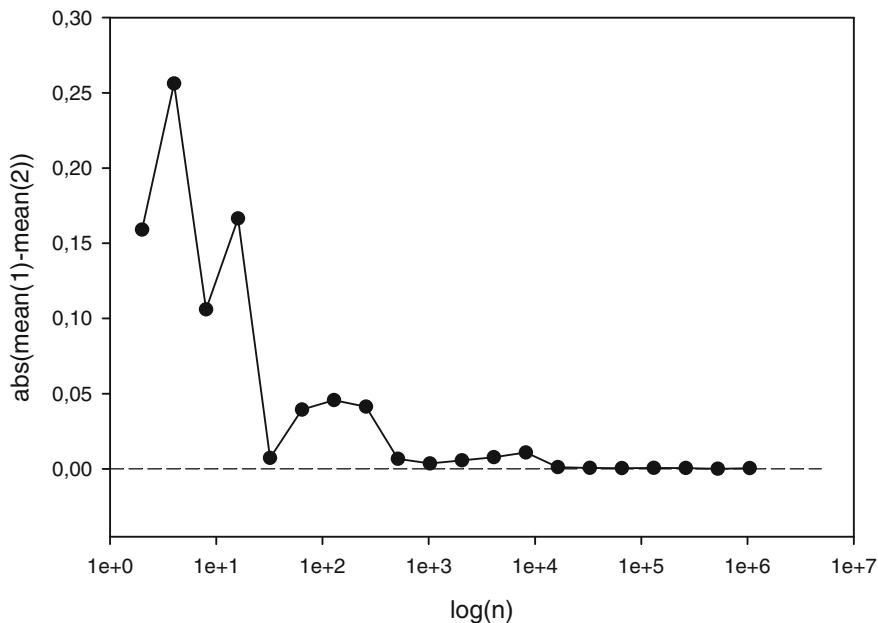
$$s_{\zeta}^2 = \frac{1}{n} \left[ \sum_{i=1}^n \zeta_i^2 - \frac{1}{n} \left( \sum_{i=1}^n \zeta_i \right)^2 \right]$$

where  $n$  is the number of micelles contained in an element of volume (Dagnelie 1980, 1981).

Figures 2.3 and 2.4 show that the difference between average values for  $\zeta$  obtained in the two volumes is significantly different up to  $10^4$  micelles per element of volume.



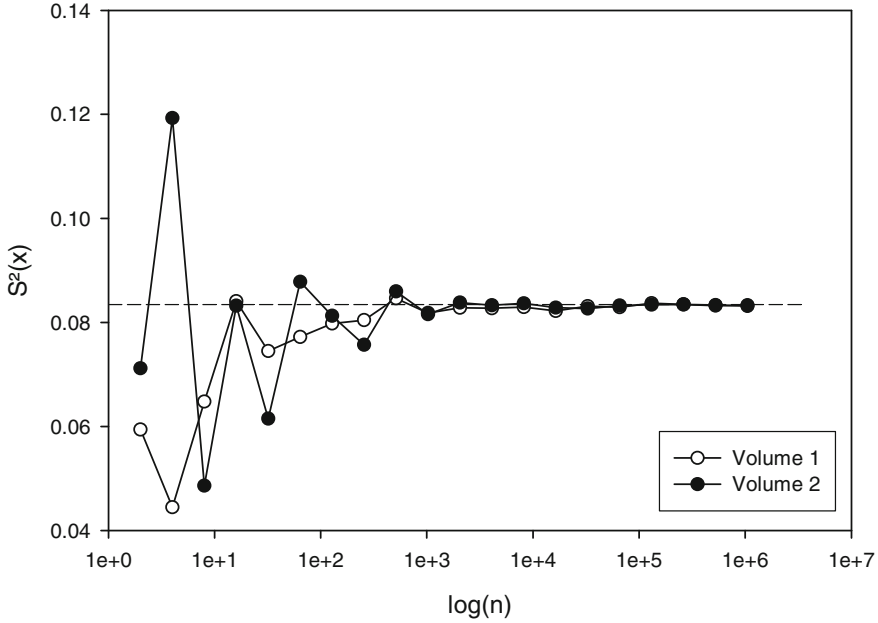
**Fig. 2.3** Means according to the number of micelles. When average values of a micellian property distributed uniformly over the interval  $[0, 1]$  in two elements of volume (1 and 2) are calculated, very different average values are noted when the number of micelles ( $n$ ) is less than or equal to 10,000. Around  $10^5$  micelles per element of volume, the two means converge toward the same value, corresponding to the theoretical value of the distribution (0.5). This value of  $10^5$  can be considered as the smallest number of micelles necessary for the average properties of the two elements of volume to be considered as statistically equal (The *dashed line* indicates the theoretical value of 0.5)



**Fig. 2.4** Absolute values of the difference between the two means. The graph shows the absolute values for the difference between the two mean values of the preceding figure. The difference decreases rapidly with the number of micelles and can be considered as zero beyond  $10^5$  micelles by element of volume

Figure 2.5 shows that variance reaches its theoretical value in the two volumes toward about  $10^5$  particles. This is a value for which the average values are about the same and very near to 0.5.

So, in the case where each micelle has the same probability of having a property of between 0 and 1 (uniform distribution), there must be  $10^5$  micelles per element of volume for two of these elements of volume, chosen at random, to have the same property average. On this basis, the order of size can be sought of the smallest element of volume that satisfies the criteria of pseudohomogeneity. Obviously, the answer is not universal and depends on the system studied. Let us take, for example, a bioreactor of 1 l in which *Escherichia coli* is being cultivated. The mass of a cell is estimated to be  $2.8 \times 10^{-13}$  g (dry weight; Neidhardt et al. 1994). Let us imagine that the total biomass in the reactor is 1 g (dry weight). The system contains  $M^c/m^c = 1/2.8 \times 10^{-13}$  some  $3.6 \times 10^{12}$  cells (obtained as the relationship between the total mass and the mass of one cell). One liter of culture contains this number of cells, and this makes it possible to calculate the volume  $\Delta V^*$  that contains  $10^5$  of them, the number necessary for pseudohomogeneity. Given that



**Fig. 2.5** Estimation of the variance according to the number of micelles. When the number of micelles is not raised to any extent, the variances of the property between the two elements of volume are different and reveal through this a greater heterogeneity in physiological states between micelles of one element of volume and another. The same applies in the case of the average (cf. Figs. 2.3 and 2.4), the variances converge toward the theoretical value (1/12; shown as *dashes*) when the number of micelles exceeds  $10^5$  by element of volume

$$\Delta V^* = \frac{V_T n^*}{N_T^c}$$

where  $V_T$  is the total volume,  $N_T^c$ , the total number of cells and  $n^*$  the number of cells necessary for pseudohomogeneity. It is found that  $\Delta V^* = 10^5 / 3.6 \times 10^{12}$ , if  $\Delta V^* = 2.8 \times 10^{-8}$  L, which corresponds to about three hundred thousandths of a milliliter. The whole system is more than 35 million times bigger than the element of volume in consideration. Moreover, the cell volume is equal to

$$v^c = \frac{m_c}{\delta_c}$$

where  $\delta_c$  is the density of a wet cell that is estimated at 1000 g/L (Dammel and Schroeder 1991; Kubitschek et al. 1983, 1984; Baldwin and Kubitschek 1984). The cell volume of a wet cell, with 70 % water (Neidhardt et al. 1994), is equal to



$2.8 \times 10^{-13} / (0.3 \times 1000)$  that comes to about  $9 \times 10^{-16}$  L. The element of volume is then 30 million times bigger than the cell. In this example, it can be incontestably confirmed that the element of volume is truly considerably greater than the micelle (cell) and sufficiently smaller than the whole system (the bioreactor) (By estimating the volume of *E. coli* to be  $4 \times 10^{-16}$  L, according to the data from Neidhardt et al. (1994), the element of volume is found to be 70 million times bigger).

If the thinking above shows that the concept of pseudohomogeneity is really applicable theoretically to suspend microorganism cultures, it is however necessary to introduce an important practical remark on the order of sizes calculated above. In fact, as has already been mentioned, the dispersed systems that are taken into consideration are unstable, in the sense that they only support themselves when dispersed if energy is brought in, in the form of agitation. The calculations above rest on the hypothesis (implied) that the system is perfectly mixed. There is not, in this case, any spatial distribution at all, any gradient, etc. It is on this one condition that the two elements of volume of the same size, taken at random can have identical average composition. Now, this condition of perfect mix, that is essentially a chemical problem, is extremely difficult to realize in practice. Numerous factors intervene (rotation speed, agitators shape, geometry of the reactor, viscosity of the medium, etc.) that makes the achievement of perfect mix very arduous. In reality, the perfect mix does not exist in a concrete system and is merely an ideal. It has been observed, in a suspension of activated sludge from a treatment plant that is subjected simultaneously to mechanical agitation (500 rpm; propeller rod stirrer) and to agitation by air bubbles (air flow of about 100 L/h through a 3 mm diameter tube, without diffuser), that there is an establishment of a vertical gradient of 0.5 gDW/l/m in a 10 l PTFE carboy with a column of water of the order of the meter in height. (The average value of biomass was 3 GDW/L.) The gradient represents a variation of about 17 % between the top and the bottom of the carboy. These remarks show just how difficult it is to master and how important the phenomena of agitation are. They also show that the element of volume calculated above is probably too small by several orders of size. Fortunately, the relationship  $V_T/\Delta V^*$  is so high that this modification of  $\Delta V^*$  remains without consequences in practice.

In conclusion, the very simplified results of the calculations confirm the hypothesis that a suspension of microorganisms can be considered conceptually as pseudohomogeneous in a device on laboratory or industrial scale. Nevertheless, when moving from the concept of an application, it is useful to make sure that all precautions are taken at the chemical engineering level to make sure that the system can be considered as a reasonably perfect mix. Unfortunately, in the literature, articles on laboratory scale give very little information on agitation in small bioreactors. When such examples will be treated, the hypothesis that the mixing is sufficient will be implied.

### 2.3 Reaction Concentration and Pseudohomogeneity

In a PDS, the micelle does not have to be homogeneous. They can be composite, structured, compartmentalized, etc. In particular, they can contain organized or soluble substances and be the site of physical and/or (bio)chemical changes, etc. The level of description on micelle level is called the local level of description. If the hypothesis that the laws of kinetics apply at this local level, is accepted, the relevant quantity of expression of these kinetics is an intensive quantity, namely the local concentration, defined by

$$C_i^p = \frac{m_i^p}{v^p} \quad (2.3.1)$$

where  $C_i^p$  is the local concentration of mass of the compound  $i$  in the micelle;  $m_i^p$  is the mass of the compound  $i$  in the micelle of phase  $p$ , and  $v^p$  is the volume of the micelle.

The local concentration is therefore a mass concentration (and not molar or any other) that is called reaction concentration or  $R$ -concentration. The  $R$ -concentrations are therefore the intensive values necessary to describe the kinetics at micellian level, but they are of little use at the system level. If the PDS is pseudohomogenous, the concept of partial pseudohomogenous concentration can be introduced and make sense (partial because it only concerns the product  $i$  in phase  $p$ ) defined by

$$\tilde{C}_i^p = \frac{1}{V_T} \sum_{i=1}^{N_T^p} m_i^p \quad (2.3.2)$$

where  $\tilde{C}_i^p$  is the partial pseudohomogenous concentration of  $i$  associated with phase  $p$  of the system;  $V_T$  the total useful volume of the system and  $N_T^p$  the total number of micelles in phase  $p$ ,  $m_i^p$  keeping the same significance as before. From a chemical point of view, (2.3.2) expresses the concentration that would be measured if, by one procedure or another, all micelles in phase  $p$  had been destroyed to liberate compound  $i$  into the global volume of the system. This is really a virtual volume that extends throughout the system. For this reason, we call it  $E$ -concentration (extended concentration).

The concentration defined by (2.3.2) is called partial because it concerns only the mass of the compound  $i$  contained in phase  $p$ . This compound can, of course, be found in other phases. There is therefore a total pseudohomogenous concentration that is obtained by adding together all phases:

$$\tilde{C}_i = \frac{1}{V_T} \sum_{j=1}^{N_p} \sum_{i=1}^{N_T^p} m_i^j \quad (2.3.3)$$

where  $N_p$  is the total number of phases. It is clear that the sum of all phases and all micelles from  $m_i$  is nothing other than the mass of the compound  $i$  in the system that is defined by

$$M_i = \sum_{j=1}^{N_p} \sum_{i=1}^{N_T^p} m_i^j \quad (2.3.4)$$

The pseudohomogenous concentration is simply,

$$\tilde{C}_i = \frac{M_i}{V_T} \quad (2.3.5)$$

This latest relationship corresponds to the usual definition of mass concentration in a homogeneous system. If  $C_i^0$  is this homogeneous concentration, it is evident that

$$C_i^0 = \tilde{C}_i \quad (2.3.6)$$

The homogenous mass concentration and the total pseudohomogenous concentration are therefore represented by the same numeric value, but the physico-chemical signification of the two quantities differs greatly. Moreover, by combining (2.3.2) and (2.3.3), it is easily shown that

$$\tilde{C}_i = \sum_{j=1}^{N_p} \tilde{C}_i^j \quad (2.3.7)$$

That simply expresses the total pseudohomogenous concentration is the sum of the partial pseudohomogenous concentrations (sum over all the phases).

### 2.3.1 Consequences of the Definition of Pseudohomogeneity

The definition of pseudohomogeneity that we have used has important consequences as regards the manner in which various quantities are calculated. We have introduced the concept of critical element of volume in such a way that two elements of volume are statistically identical. This choice allows the definition of significant average values from the center of an element of volume and the elimination of the necessity of using a structured representation of micelles. Let us take a simple example. The time that passes between the moment of cell division and the moment in mind is called “cell age.” It is evident that in each element of volume, there are cells of different ages, the ages being distributed according to a certain statistical law. The models that take into account this type of distribution are called structured (refer, for example, to Fredrickson and Tsuchiya 1963,

Tsuchiya et al. 1966, Minkevitch and Abramychiev 1994, Schügerl and Bellgardt 2000). In our approach, the elements of volume are chosen so that the average age of the cells is the same in all elements of volume and the same goes for all micellian properties. In fact, it is considered that in an element of volume of convenient size, all cells are identical and characterized by average values. Let us examine the impact of this point of view on the following relationship

$$M_i^p = \sum_{i=1}^{N_T^p} m_i^p \quad (2.3.8)$$

that expresses that the whole mass of the compound  $i$  in phase  $p$  is equal to the sum of the masses of this compound contained in all micelles of the phase. This relationship is evidently always true, whatever the distribution of the compound in the various micelles. If it is now considered that all micelles are statistically identical  $m_i^p$  is then the average value of the compound in an average micelle. It can then be written that if  $N_T^p$  is the total number of micelles in the phase  $p$ , that

$$M_i^p = N_T^p m_i^p. \quad (2.3.9)$$

Just the opposite holds in (2.3.8), this relationship is only true in certain conditions that are exactly those that have been defined for the critical element of volume and the criteria for pseudohomogeneity.

This approach can be applied to various quantities, thus the volume of phase  $p$  will be

$$V^p = N_T^p v^p \quad (2.3.10)$$

That is to say  $N_T^p$  times the micellian volume  $v_p$ .

### 2.3.2 Phase Density

By definition phase density shall be called the quantity,

$$X^p = \frac{M^p}{V_T} = \frac{N_T^p m^p}{V_T} \quad (2.3.11)$$

That is the relationship of the total mass of phase  $p$  to the total useful volume.

According to custom, the term, “biomass” will be kept for this quantity when the micelles that are in mind in (2.3.11) are cells.

### 2.3.3 Phase Volume Relationship

Equation (2.3.11) can be rewritten as

$$N_T^p = \frac{X^p V_T}{m^p} \quad (2.3.12)$$

and (2.3.10) put in the form

$$N_T^p = \frac{V^p}{v^p} \quad (2.3.13)$$

By equalizing these two equations and rearranging, the following is found,

$$\frac{V^p}{V_T} = \frac{X^p}{\delta_p} \quad (2.3.14)$$

or, by definition,

$$\delta_p = \frac{m^p}{v^p} = \frac{M^p}{V^p} \quad (2.3.15)$$

is the mass volume of an average micelle or of a phase.

### 2.3.4 Relationship Between *R*- and *E*-Concentrations

Relationships above are going to make it possible to calculate the relationship between the *R*- and the *E*-concentrations.

By definition, the *R*-concentration is (cf. (2.3.1))

$$C_i^p = \frac{m_i^p}{v^p} = \frac{M_i^p}{V^p} \quad (2.3.16)$$

By multiplying the top right and bottom right of the equation by  $V_T$  and using (2.3.2), (2.3.8) and (2.3.14), it is easily shown that

$$C_i^p = \tilde{C}_i^p \frac{\delta_p}{X^p} \quad (2.3.17)$$

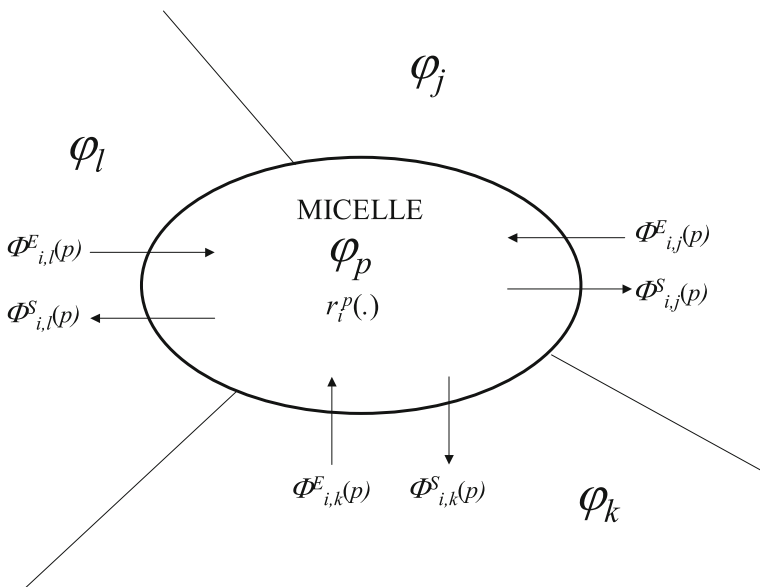
This equality shows the relationship that exists between the local reaction level (the *R*-concentrations) and the extended concentrations (*E*-concentrations). It is not directly implied that the phase density intervenes as a factor. This relationship (and its consequences) will be abundantly used in the application of explicit forms of mass balances of PDS (cf. Chap. 3, Sect. 3.2.3).

## 2.4 Mass Balances

Let us consider a micelle from phase  $p$  encircled by three phases  $\varphi_j, \varphi_k, \varphi_l$ , as represented in Fig. 2.6.

The micelle that belongs to phase  $p$  is an open system (exchanging energy and matter with each of the phases). Let us assume that each phase contains a constituent  $P_i$ , represented by its index  $i$ . The transfer flux of the compound  $i$  (expressed as a unit of mass of the compound per unit of time, for example, in g/h) is represented by  $\phi_{i,q}^x(p)$ , where  $i$  is the index for the transferred compound,  $q$ , the index for the phase of origin and  $p$  that of the phase concerned (that of the micelle). The exponent  $x$  can take value  $E$  or  $S$  and these indicate whether the compound is entering or leaving the micelle. Moreover, the compound can undergo changes (biochemical, physical, etc.) inside the micelle. These changes are represented by the reaction rate per unit of volume,  $r_i^p(\cdot)$  where  $i$  is the reacting compound index,  $p$  the phase where the change is taking place;  $(\cdot)$  indicates that the kinetic  $r$  is a complex function of several variables (concentrations, kinetic coefficients, temperature, pH,...). In a general way,  $r_i^p(\cdot)$  is a resulting kinetics which can be broken up into several terms (possibly with different signs):

$$r_i^p(\cdot) = \sum_m r_{i,m}^p(\cdot) \quad (2.4.1)$$



**Fig. 2.6** Exchange and transformation terms. The micelle in phase  $p$  can exchange product  $i$  with the three surrounding phases  $j$ ,  $k$ , and  $l$ . Transformation reactions  $r_i^p(\cdot)$  take place within the micelle

On the micelle level, kinetics depend (among others) on local concentrations, that is to say, the  $R$ -concentrations. Formally,

$$r_i^p(.) = (\{C_n^p\}, \dots) \quad (2.4.2)$$

In accordance with usual kinetics, the units of  $r$  are expressed in mass units per unit of time and per unit of micellian volume (for example, in g/(h L) or in g/(h  $\mu\text{m}^3$ )).

The mass balance of the compound  $i$  at the center of the micelle is written as

$$\frac{dm_i^p}{dt} = \sum_{q=1; q \neq p}^{N_p} [\phi_{i,q}^E(p) - \phi_{i,q}^S(p)] + r_i^p(.)v^p \quad (2.4.3)$$

where  $m_i^p$  is the mass of the compound  $i$  in the micelle and  $v^p$  is the average volume of the micelle. The first term of the right side represents the total transfer flows in all phases ( $N_p$  is the number of phases).

In order to simplify the written formula, it is written as,

$$\varphi_{i,\{q \neq p\}}(p) = \sum_{q \neq p} [\phi_{i,q}^E(p) - \phi_{i,q}^S(p)] \quad (2.4.4)$$

This quantity represents the net interphasic exchange flux of the compound  $i$  at the level of the micelle. The balance (2.4.3) is written as,

$$\frac{dm_i^p}{dt} = \varphi_{i,\{q \neq p\}}(p) + r_i^p(.)v^p \quad (2.4.5)$$

From the point of view taken, the balance at micelle level is not useful; a more macroscopic description will be sought. Let us begin by writing the mass balance for the total number of micelles that make up phase  $p$ .

Let  $N_T^p$  be the number of micelles that make up the phase. Since all micelles are statistically identical, simply multiply the left and right side of Eq. (2.4.5) by  $N_T^p$ ; therefore,

$$N_T^p \frac{dm_i^p}{dt} = N_T^p \varphi_{i,\{q \neq p\}}(p) + N_T^p r_i^p(.)v^p \quad (2.4.6)$$

$N_T^p v^p$  is nothing other than the volume of the phase,

$$V^p = N_T^p v^p \quad (2.4.7)$$

Moreover,

$$\Phi_{i,\{q \neq p\}}(p) = N_T^p \phi_{i,\{q \neq p\}}(p) \quad (2.4.8)$$

The total mass of the compound  $i$  for all the micelles of this phase is simply

$$M_i^p = N_T^p m_i^p \quad (2.4.9)$$

In a general case, the number of micelles is not constant and the derivative with respect to time of (2.4.9) is,

$$\frac{dM_i^p}{dt} = N_T^p \frac{dm_i^p}{dt} + m_i^p \frac{dN_T^p}{dt} \quad (2.4.10)$$

By using the relationships, (2.4.7) to (2.4.10) in (2.4.6), the mass balance of the product  $i$  over the group of micelles is written as,

$$\frac{dM_i^p}{dt} = \Phi_{i,\{q \neq p\}}(p) + r_i^p(\cdot) V^p + m_i^p \frac{dN_T^p}{dt} \quad (2.4.11)$$

By dividing and multiplying the last term of the right side by  $N_T^p$  and by using the logarithmic derivative, it is finally found that

$$\frac{dM_i^p}{dt} = \Phi_{i,\{q \neq p\}}(p) + r_i^p(\cdot) V^p + M_i^p \frac{d \ln N_T^p}{dt} \quad (2.4.12)$$

It could be believed that the balance (2.4.12) truly is the required expression of the phase balance. However, it should be mentioned that nothing has been said about the real apparatus in which the phases and micelles are contained. In reality, the balance (2.4.12) is complete for a closed apparatus; it is incomplete for an open apparatus. Since the main interest is in an open system (as the chemostat), it is convenient to complete the balance. The incompleteness of (2.4.12) is not, however, immediately taken notice of nor implied, because the variational term for the number of micelles ( $d \ln N_T^p$ ) leaves it to be assumed that the system is open. It is no such thing, in fact, because this term appears only as the variation in micelles containing the compound  $i$ . In reality, the balance is closed in relation to the compound, in spite of the variational term of micelles. In fact, the term  $d \ln N_T^p$  does not represent an inflow/outflow term for micelles, but only expresses the combining aspect of the interphasic exchange process of  $i$  and the way the compound is distributed between the different micelles.

To complete the balance, the level of description chosen should be above that for the micelle and should include the entire system.



At system level, the variation in mass of the compound can be written as,

$$\frac{dM_i}{dt} = F_i^E - F_i^S + \sum_p \text{contribution of the micella} \quad (2.4.13)$$

where  $F_i^E$  and  $F_i^S$  are global terms for inflow of  $i$  via one or several phases (including possibly the dispersing phase). The sum of the contribution of the micelles is calculated for all phases (it being understood that phases that do not contain  $i$  have no contribution).

By using (2.4.12), it is found that

$$\frac{dM_i}{dt} = F_i^E - F_i^S + \sum_p \left[ \Phi_{i,\{q \neq p\}}(p) + r_i^p(.)V^p + M_i^p \frac{d \ln N_T^p}{dt} \right] \quad (2.4.14)$$

The term  $\Phi_{i,\{p \neq q\}}(p)$  represents the net transfer flow of the contributions from all phases of phase  $p$ . It is evident that the same quantity of  $i$  that enters into phase  $p$  leaves phases  $q \neq p$ . It is obvious that the reasoning applied to phase  $p$  can also be applied to all other phases. Ultimately, it is easy to understand that, at the level of the whole system, the interphasic transfer fluxes are canceled out, since all quantities of matter that exit a phase are necessarily compensated for by the inflow of matter in other phases and vice versa. So,

$$\sum_{p=1}^{N_p} \Phi_{i,\{q \neq p\}}(p) \equiv 0 \quad (2.4.15)$$

(A stricter equation is given as evidence in Appendix A.4.) In equation form, (2.4.14) is reduced to

$$\frac{dM_i}{dt} = F_i^E - F_i^S + \sum_p \left[ r_i^p(.)V^p + M_i^p \frac{d \ln N_T^p}{dt} \right] \quad (2.4.16)$$

This relationship expresses the variation in mass of the compound  $i$  within the whole system. It includes, thanks to the inflow/outflow terms, the possibility of modifying arbitrarily, the number of micelles in each phase.

Before seeking the complete balance at the level of phase, this mass balance (in extensive form) will be expressed in an intensive form, that is to say, in terms of concentrations. For this, let us divide the left and right sides of (2.4.16) by  $V_T$ :

$$\frac{1}{V_T} \frac{dM_i}{dt} = \frac{F_i^E - F_i^S}{V_T} + \sum_p \left[ r_i^p(.) \frac{V^p}{V_T} + \frac{M_i^p}{V_T} \frac{d \ln N_T^p}{dt} \right] \quad (2.4.17)$$

In a general case  $V_T$  is not a constant and

$$\frac{d}{dt} \left( \frac{M_i}{V_T} \right) = \frac{1}{V_T} \frac{dM_i}{dt} - \frac{M_i}{(V_T)^2} \frac{dV_T}{dt} \quad (2.4.18)$$

So,

$$\frac{d}{dt} \left( \frac{M_i}{V_T} \right) = \frac{F_i^E - F_i^S}{V_T} + \sum_p \left[ r_i^p(\cdot) \frac{V^p}{V_T} + \frac{M_i^p}{V_T} \frac{d \ln N_T^p}{dt} \right] - \frac{M_i}{V_T} \frac{d \ln V_T}{dt} \quad (2.4.19)$$

By using the definitions (2.3.1) and (2.3.5) for concentrations (2.4.19) it can be written as

$$\frac{d\tilde{C}_i}{dt} = \frac{F_i^E - F_i^S}{V_T} + \sum_p \left[ r_i^p(\cdot) \frac{V^p}{V_T} + \tilde{C}_i^p \frac{d \ln N_T^p}{dt} \right] - \tilde{C}_i \frac{d \ln V_T}{dt} \quad (2.4.20)$$

Taking into account that the total pseudohomogenous concentration is the sum of the partial pseudohomogeneous concentrations (2.3.7), (2.4.20) is expressed in terms of partial concentrations,

$$\sum_p \frac{d\tilde{C}_i^p}{dt} = \frac{F_i^E - F_i^S}{V_T} + \sum_p \left[ r_i^p(\cdot) \frac{V^p}{V_T} + \tilde{C}_i^p \frac{d \ln N_T^p}{dt} \right] - \sum_p \tilde{C}_i^p \frac{d \ln V_T}{dt} \quad (2.4.21)$$

So it is easy to write the equation for each of the phases  $p$  by distributing properly the terms of (2.4.21):

$$\frac{d\tilde{C}_i^p}{dt} = \frac{\tilde{F}_i^{p,E} - \tilde{F}_i^{p,S}}{V_T} + r_i^p(\cdot) \frac{V^p}{V_T} + \tilde{C}_i^p \left( \frac{d \ln N_T^p}{dt} - \frac{d \ln V_T}{dt} \right) + FE_i^p \quad (2.4.22)$$

The first term of the right side of (2.4.22) expresses the distribution of the inflows of each phase. These flows are expressed in terms of pseudohomogenous concentration from which the formulae are derived (tilde). The latest term of the right side expresses the interphasic exchange flows that appear when the system is divided into its distinctive phases.

$FE_i^p$  is nothing other than the interphasic exchange term divided by the useful volume, namely,

$$FE_i^p \equiv \frac{\Phi_{i,\{p \neq q\}}(p)}{V_T} \quad (2.4.23)$$

and

$$\Phi_{i,\{p \neq q\}}^0(p) \equiv \frac{\Phi_{i,\{p \neq q\}}(p)}{V_T} \quad (2.4.24)$$

That is called the interphasic exchange flux by unit of volume.

By using the volumic relationship of phase (2.3.14) and writing,

$$q_i^p = \frac{r_i^p(\cdot) V^p}{M^p} \quad (2.4.25)$$

The mass balance of the phase (2.4.22) is obtained in the following intensive form,

$$\frac{d\tilde{C}_i^p}{dt} = \frac{\tilde{F}_i^{p,E} - \tilde{F}_i^{p,S}}{V_T} + q_i^p X^p + \Phi_{i,\{q \neq p\}}^0(p) + \tilde{C}_i^p \left( \frac{d \ln N_T^p}{dt} - \frac{d \ln V_T}{dt} \right) \quad (2.4.26)$$

The left side expresses the time variation in pseudohomogenous concentration (*E*-concentration) of the compound *i* in phase *p* (unit: mass/(volume.time)). On the right side of (2.4.26):

- The first term expresses, in *E*-concentrations, the inflow and outflow terms of the compound *i* contained in phase *p* at the level of the global system. These are therefore inflow/outflow terms associated with the micelles of phases *p* (units: mass/(volume.time));
- The second term is the product of density of phase by the net kinetics of the changes in the compound at the center of the micelles of phase *p*. The phase density is essentially a mass concentration (mass/volume). Since  $q_i^p$  being speed per unit of phase density, it is in reality a specific speed (units: time<sup>-1</sup>);
- The third term is the net interphasic exchange flow (units: mass/(vol temps). It represents the net transfer flux of compound *i* stemming from or leaving all other phases;
- The fourth term is a variational term that takes into account the combined variation of the number of micelles in the phase and the variation in the useful volume. For a system of constant volume, this latest contribution is obviously nil.

*Note:* It appears clearly in this section that the decomposition of a global system into its different phases (Eq. 2.4.22) makes the interphasic exchange flows appear and these are not reduced to equations in a description based only at the level of the system.

### 2.4.1 *Note Concerning the Variational Term Appearing in (2.4.12)*

In order to keep as general a character as possible, it is assumed that the number of micelles was not constant at the time of the derivation of (2.4.9). This introduces the number of micelles time derivative and is called the variational term. It is important to understand the import of this term to avoid misinterpretation in the future, mainly at the time of derivation of the rate of everything (refer to Appendix A.3). To remind us, in the process of derivation of (2.4.12), it was begun with a mass balance at the level of the micelle to construct, by addition, the balance at the level of the phase. Variation in the number of micelles included in the variational term is therefore a variation at the phase level only and does not represent an inflow/outflow term at the global level of the whole system. Even more important, the variational term does not represent the variation in the number of micelles by division that cell division alone produces in general, nor by cell division in particular. In fact, it is easy to understand that cell division neither produces nor consumes any compounds at all (except a minimum part linked to the energy necessary for the division). A representation of cell division alone cannot be brought in as a source or sink term for a balance such as (2.4.10). In short, it is known that the variational term does not exist for it is neither a term of exchange with the exterior world, nor a term representing an aggregation or the division of micelles (p. ex. cell division). In reality, this term represents an interphasic exchange term, that is to say, the inflow or the outflow of micelles in phase  $p$  and stemming from one (or several) other phases. Let us consider a concrete example. Let us imagine a solid phase composed of two cell compartments, namely inactive cells  $A$  and active cells  $A^*$ . If an activation/inactivation process of the type  $A \rightleftharpoons A^*$  exists, account must be taken of this variation in the number of micelles in phase  $p$  and in phase  $p^*$ . It is this type of situation that takes into account the variation term that appears in (2.4.10) and (2.4.12). If there is only one single cell phase, a cell viability rate of 100 % implies that the variational term is zero; if there is cell mortality, the variational term is necessarily negative, implying a loss of micelles from phase  $p$  by cell death. In this latter case, there is no interphasic exchange of micelles to speak of since there is only one micellian phase, but there is nevertheless an exchange flow for the dispersing phase. (If this state of affairs is annoying as regards formulae, it is always possible to create a compartment of dead cells and so generate a second micellian phase.)

A slightly different way of approaching this delicate problem is described in Appendix A.5.

## 2.5 The Grouping Principle

Equation (2.4.26) is valid for a specific compound. However, it is sometimes desirable to consider groups of compounds (or families) of particular interest.

Let  $NC^p$  be the total number of different constituents of phase  $p$  and let  $NF^p$  be the number of families of compounds from this same phase ( $NF^p \leq NC^p$ ). Now let  $NC_k^p$  be the number of different compounds belonging to the family (or group)  $k$  from the phase  $p$  ( $NC^p = \sum_k NC_k^p$ ).

Since the  $E$ -concentrations are additional, a partial, pseudohomogenous concentration can be defined by,

$$\tilde{C}_k^p = \sum_{i=1}^{NC_k^p} \tilde{C}_i^p; \quad i \in \mathfrak{S}_k \quad (2.5.1)$$

where  $\mathfrak{S}_k$  represents the family or the group  $k$ . So,

$$\frac{d\tilde{C}_k^p}{dt} = \sum_{i=1}^{NC_k^p} \frac{d\tilde{C}_i^p}{dt}; \quad i \in \mathfrak{S}_k \quad (2.5.2)$$

and the laws of evolution, themselves, are also additive.

Consequently, the system of equations  $NC^p$  in each phase  $p$  can be replaced by a reduced system of equations  $NF^p$  in the form,

$$\frac{d\tilde{C}_k^p}{dt} = \frac{1}{V_T} \sum_{i=1}^{NC_k^p} (\tilde{F}_i^E - \tilde{F}_i^S) + X^p \sum_{i=1}^{NC_k^p} q_i^p + \sum_{i=1}^{NC_k^p} \Phi_{i,\{q \neq p\}}^0(p) + \tilde{C}_k^p \left( \frac{d \ln N_T^p}{dt} - \frac{d \ln V_T}{dt} \right) \quad (2.5.3)$$

which is written in an identical form in (2.4.26)

$$\frac{d\tilde{C}_k^p}{dt} = \frac{1}{V_T} (\tilde{F}_k^E - \tilde{F}_k^S) + q_k^p X^p + \Phi_{k,\{q \neq p\}}^0(p) + \tilde{C}_k^p \left( \frac{d \ln N_T^p}{dt} - \frac{d \ln V_T}{dt} \right) \quad (2.5.4)$$

with

$$q_k^p = \sum_i q_i^p; \quad \tilde{F}_k^E - \tilde{F}_k^S = \sum_i (\tilde{F}_i^E - \tilde{F}_i^S); \quad \Phi_{k,\{q \neq p\}}^0(p) = \sum_i \Phi_{i,\{q \neq p\}}^0(p) \quad (2.5.5)$$

In other terms, the evolution law for a family (or a group) of compounds is strictly isomorphic to the evolution law of an isolated compound. This result is particularly interesting in the sense that it makes it possible to introduce several levels of description for the center of the system, using the same formulae. The evolution of the system can then be followed at the metabolite level or just that the

level of groups of metabolites (by family, group, or metabolic pathway) or by combinations of each of these levels of description.

## 2.6 Comment on a Certain Relativity in Writing the Mass Balance

It can be quite annoying to note that it is necessary to use two levels of description (micellian and systemic) to obtain the correct and complete expression of the balance in a phase.

On one hand, if one starts at the micellian level of description and if we sum on them, the phase balance is given by (2.4.12). The balance of the system is then given by the sum of the phase balances over all the phases.

On the other hand, by starting at the system level of description, the phase balance is obtained by decomposition of the whole system into its various phases.

What is remarkable is that it is by using these two methods that are apparently symmetrical, the results obtained are not the same.

Let us quickly go over the development again by distinguishing the levels of description used. The relationship (2.4.14) can be put in the form,

$$\left. \frac{dM_i}{dt} \right|_{\text{SYS}} = F_i^E - F_i^S + \left. \frac{dM_i}{dt} \right|_{\text{mic}} \quad (2.6.1)$$

The notation  $|_X$  indicates that the level of description used to derive the expression (“level X” in the example).

By dividing the left and right sides of (2.6.1) by a constant volume  $V_T$ , it is obtained that

$$\left. \frac{d\tilde{C}_i}{dt} \right|_{\text{SYS}} = \frac{F_i^E - F_i^S}{V_T} + \left. \frac{d\tilde{C}_i}{dt} \right|_{\text{mic}} \quad (2.6.2)$$

The phase balance obtained from micelles is given by (2.4.12); all that needs to be done is to add up all the phases to obtain the contribution at the level of the system. So,

$$\left. \frac{d\tilde{C}_i}{dt} \right|_{\text{mic}} = \sum_p \left. \frac{d\tilde{C}_i^p}{dt} \right|_{\text{mic}} \quad (2.6.3)$$

On one hand, (cf. (2.3.7))

$$\tilde{C}_i = \sum_p \tilde{C}_i^p \quad (2.6.4)$$

and on the other hand, the global inputs/outputs of  $i$  can be distributed between the different phases,

$$F_i^E - F_i^S = \sum_p \left( \tilde{F}_i^{p,E} - \tilde{F}_i^{p,S} \right) \quad (2.6.5)$$

Using (2.6.5) in (2.6.2), it is obtained that

$$\sum_p \left. \frac{d\tilde{C}_i^p}{dt} \right|_{\text{SYS}} = \sum_p \frac{F_i^{p,E} - F_i^{p,S}}{V_T} + \sum_p \left. \frac{d\tilde{C}_i^p}{dt} \right|_{\text{mic}} \quad (2.6.6)$$

It is easy to distribute the terms of the sum by letting each affect a phase and so,

$$\left. \frac{d\tilde{C}_i^p}{dt} \right|_{\text{SYS}} = \frac{F_i^{p,E} - F_i^{p,S}}{V_T} + \left. \frac{d\tilde{C}_i^p}{dt} \right|_{\text{mic}} \quad (2.6.7)$$

where the latest term from the right side is no other than (2.4.12) divided by  $V_T$ . It is shown that

$$\left. \frac{d\tilde{C}_i^p}{dt} \right|_{\text{mic}} = \Phi_{i,\{p \neq q\}}^0(p) + r_i^p(\cdot) \frac{V^p}{V_T} + \tilde{C}_i^p \frac{d \ln N_T^p}{dt} \quad (2.6.8)$$

Let us write, (2.6.7) in the more compact form,

$$v(i)|_{\text{SYS}} = v_{E/S}(i) + v(i)|_{\text{mic}} \quad (2.6.9)$$

where  $v(i)|_{\text{SYS}}$  and  $v(i)|_{\text{mic}}$  are rates of changes in  $i$  obtained, respectively, by a level of description at the level of the system and the micelle,  $v_{E/S}(i)$  is the net inflow/outflow speed of the system. By analogy, the term “referential” can be used rather than level of description. It is then noted that the speed of (bio)chemical change of the compound  $i$  in phase  $p$  depends on the referential used when writing the equations. This proposition is to be matched with this: “*In different basic systems the laws governing movement do not generally take the same form.*” (Landau and Lifchitz 1969), that is a basic proposition in the Galilean principle of relativity.

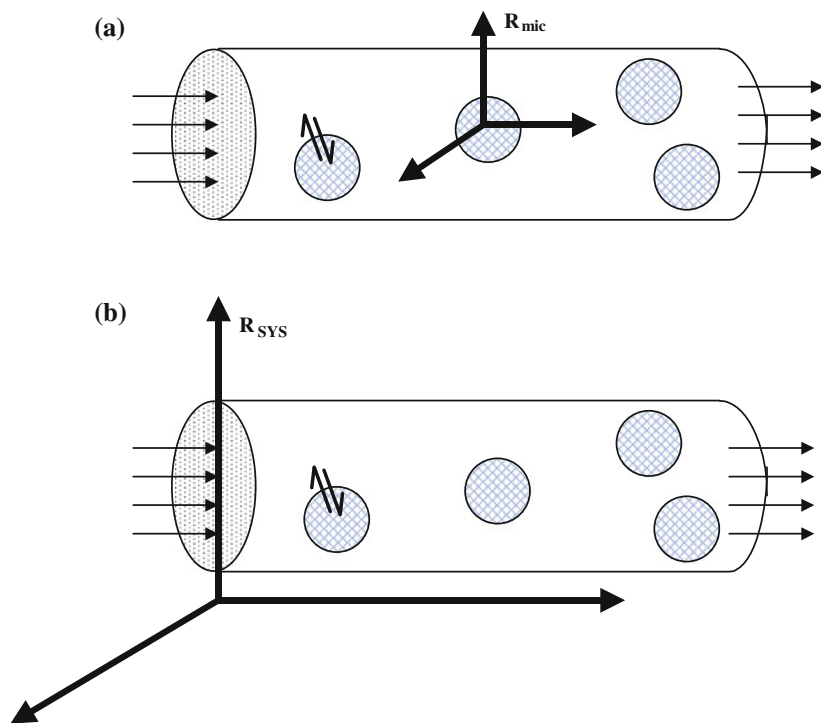
The important point about this principle of kinetic relativity (PKR) is that the relativity of the observed rates depends on whether or not the system is open. In fact, if the system is closed,  $v_{E/S}(i) \equiv 0$  and so from (2.6.9):

$$v(i)|_{\text{SYS}} = v(i)|_{\text{mic}} \quad (2.6.10)$$

So the choice of referential makes no difference.

### 2.6.1 Discussion

It is not thought that this principle of kinetic relativity or PKR is of just small import from the point of view of current practice and of mainly the modeling of cell mechanics and/or bioreactors. In the first case, it happens frequently that cell physiological models are developed without specifying which environment the cell is in (batch, fed-batch, chemostat, etc.). It is possible to write a model in these conditions, but it is not possible to complete experimental verification without choosing a particular environment for the cell. The PKR can then cause discordances between the model and experimental verification, to appear simply because the ways in which the model is obtained belong to one referential, whereas the experimental verification belongs to another. Just the same, the observation of state variables at the level of a bioreactor can lead to false deductions about intracellular mechanisms if possible corrections due to PKR are ignored. Figure 2.7 schematizes the situation that leads to the relative speeds being obtained (2.6.9).



**Fig. 2.7** Representation of the referentials. The *horizontal cylinder* represents an open system in which a flow causes micelles containing the compound  $i$  to get in and out. In case **a**, the kinetics of the transformation in  $i$  is obtained by fixing the referential on one micelle ( $R_{mic}$ ). In case **b**, the kinetic is obtained by working within the referential of the whole system ( $R_{sys}$ ). The results obtained are different and linked by the relationship (2.6.9). By analogy with the mechanics, in  $R_{mic}$  the micelles is “at rest” (corresponds to a closed system), whereas in  $R_{sys}$ , the micelle is “in motion” (corresponds to an open phase)



### 2.6.2 Example

To reinforce the considerations above of the possibility of a PKR, a very simple example of relativity based on a more intuitive than mathematical approach is proposed.

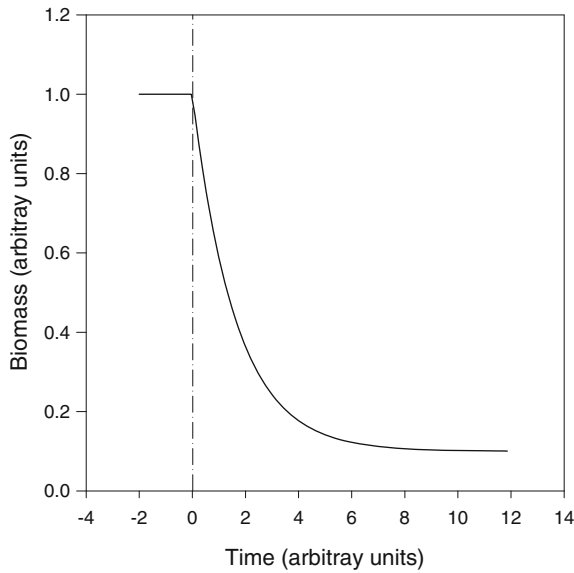
Figure 2.8 shows the development of biomass  $X$  (cell phase density) during the sudden increase in the dilution rate in a chemostat previously at steady state.

For  $t < 0$ , a steady state has been reached where  $D = D_1$ ; at  $t = 0$ , there is a sudden shift up caused by rapidly increasing  $D$  to  $D_2$ , with  $D_2 > D_1$ . A transient regime follows that is characterized by a decrease in biomass, and this regime tends toward another stationary state (if  $D_2$  does not exceed the washout value).

Let us assume that there is an analytical expression,  $X(t)$ . In fact, in this example, a plausible arbitrary expression has been used (inspired by data described in Thierie et al. 1999):

$$X(t) = X(0) [e^{-D_2 t} + b(1 - e^{-kt})] \quad (2.6.11)$$

where  $b = 0.1$  and  $k = 0.5$ ;  $D_2 = 0.6$  et  $D_1 = b k = 0.05$  (2.6.11) is a positive, decreasing monotone function.



**Fig. 2.8** Development in biomass after a dilution rate shift up. A steady state has been reached in the chemostat (time  $< 0$ ) for a given dilution rate ( $D_1$ ) when there is a sudden increase at time  $t = 0$ . The biomass decreased rapidly and relaxed to another steady state, corresponding to dilution rate  $D_2$

At the start of such data, the only way to calculate the specific growth rate,  $\mu$  is to calculate the ratio between the rate (time derivative) and the biomass, let

$$\mu = \frac{X'(t)}{X(t)} \quad (2.6.12)$$

A negative specific speed of growth is obtained, since (2.6.11) is a monotonously decreasing function.

In reality, (2.6.12) is only the expression of net rate obtained at the system level and it should be written as

$$\mu|_{\text{sys}} = \frac{X'(t)}{X(t)} \quad (2.6.13)$$

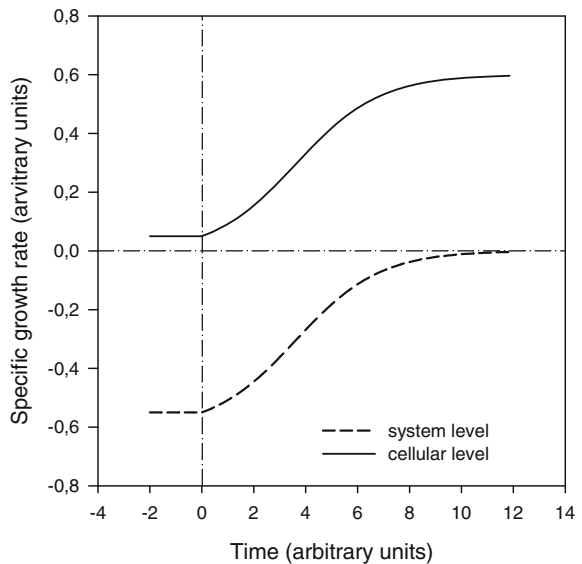
which is represented as dashes in Fig. 2.9.

The relationship (2.6.13) represents, in fact the balance between cell growth and the outflow of cells from the chemostat. So it is seen that the terms of inflow/outflow appear and thus intervene in the principle of kinetic relativity.

The passive outflow term can easily be evaluated, that is to say the outflow speed of cells in the absence of their growth. In a perfectly agitated system, the outflow speed will be equal to the dilution rate multiplied by the biomass in the reactor, let

$$\frac{dX}{dt} = -D_2 X \quad (2.6.14)$$

**Fig. 2.9** Relative rates. The specific rate observed in the system referential (*in dashes*) is negative and tends toward zero, whereas the specific growth rate of the cells (micellian referential) is positive and tends toward its steady value,  $D_2$



By rearranging, the specific speed is obtained,

$$\frac{1}{X} \frac{dX}{dt} = -D_2 \quad (2.6.15)$$

so, by definition,

$$\mu_{E/S} = -D_2 \quad (2.6.16)$$

By using (2.6.9), it is then possible to calculate the specific speed of growth of the cells (in the micellian referential),

$$\mu|_{\text{cell}} = \mu|_{\text{SYS}} - \mu_{E/S} \quad (2.6.17)$$

So by using (2.6.13) and (2.6.16)

$$\mu|_{\text{cell}} = \frac{X'(t)}{X(t)} + D_2 \quad (2.6.18)$$

This value appears as a solid line in Fig. 2.9 and is now a positive quantity that tends clearly toward  $D_2$  when  $t$  tends to infinity. When  $t$  tends to infinity, (2.6.18) tends toward the value,

$$\mu|_{\text{cell}} \approx D_2 \quad (2.6.19)$$

In order to take up again the kinetic relativity illustrated here in a naïve way, the following two equivalent propositions can be given,

- (a) “The specific growth rate of the biomass tends toward zero in the chemostat.” This expresses what is observed in the referential of the system and it is nothing other than the steady state of the system.
- (b) “The specific growth rate of the cells tends toward  $D_2$  for the new steady state.” This is the result of the calculation that gives the speed in the micellian referential.

It is evident that the two propositions are true, but that the values attributed to the specific growth rates are different and depend on the referential (level of description) in which they are described.

## 2.7 Influence of Phase Density on Concentrations Calculation

Simple theoretical and experimental studies have, since the 1970s, shown that increase in biomass could improve productivity of continuous cultures (Monbouquette 1987). Later, it was realized that continuous techniques using high

cell densities, represented a good method for avoiding cell intoxication phenomena due to release of toxic by-products. (Posten and Rinas 2000).

Walker (Walker 1998) distinguishes high cell density cultures (HCDC: *high cell density cultivation*) of which the biomass is greater than 50 g/L (dry weight, DW) and very high density cell cultures (VHCDC) that present a biomass greater than 150 gDW/L. By examining the constraints linked to such cultures (oxygenation, agitation, etc.), he quotes the example of a culture reaching DW/L that Chen et al. (1997) worked on.

So, there are production processes that use very high cell phase densities (biomass). In a representation of polyphasic dispersed systems, there is a difference between *R*- and *E*-concentrations (refer to Sect. 2.3) and this use of one or another concentration must be made sure of with particular care. However, the treatment of a problem can sometimes be very much simplified if the two concentrations are very similar and can be mixed one with another without introducing significant error (during experimentation, for example). In this section, the conditions necessary for using one or another concentration interchangeably were examined.

The experimental concentrations are, in general, *R*-concentrations. So the dose of a compound *i* from the culture medium, obtained by taking samples from the reactor that are measured after filtration, is expressed through a homogenous concentration obtained in the liquid phase (for example). So the quantity of compound is determined in an aliquot portion that has been made monophasic (here, by filtration). The concentration determined in the aliquot can be spread through the concentration in the corresponding phase of the system. However, it cannot be spread throughout the whole system without introducing error, if account is not taken of the density of the other phases. In fact, the working volume of the reactor is the sum of all the phase volumes. By mixing the volume of one single phase with the working volume, an error is made that in certain cases can be significant. A contrario, it is not always justifiable to devote oneself to complicated calculations to improve a value for which the experimental error is much greater than the correction made on volume.

Let us consider a concentration obtained experimentally in phase *p*; this is an *R*-concentration calculated by,

$$C_i^p = \frac{M_i^p}{V^p} \quad (2.7.1)$$

Moreover, the partial, pseudohomogenous concentration is given by,

$$\tilde{C}_i^p = \frac{M_i^p}{V_T} \quad (2.7.1)$$

By eliminating  $M_i^p$  from the two relationships and rearranging, it is easily found that

$$\tilde{C}_i^p = C_i^p \frac{V^p}{V_T} \quad (2.7.3)$$

Since the total volume is greater than the phase volume, it is obviously observed that the  $E$ -concentration is less than the  $R$ -concentration.

Taking into account that the total volume is the sum of all phase volumes,

$$V_T = V^p + \sum_{k \neq p} V^k \quad (2.7.4)$$

Equation (2.7.3) can be put in the form,

$$\tilde{C}_i^p = C_i^p \frac{V^p}{V^p + \sum_{k \neq p} V^k} \quad (2.7.5)$$

It is clear that

$$V^p \gg \sum_{k \neq p} V^k \quad (2.7.6)$$

The two concentrations have a value that is very similar and

$$\tilde{C}_i^p \approx C_i^p \quad (2.7.7)$$

Condition (2.7.6) is the general condition that makes it possible to use either of the two concentrations by introducing just one minimum or insignificant error. So it suffices that the volume of the phase in consideration is much greater than the sum of all the other phases. This form is, however, generally very impractical and it is hoped that a less general condition that is much more helpful in practice will be introduced.

### 2.7.1 Two-Phase System

For this, let us consider a two-phase system: solid and liquid. The relationship (2.7.5) is reduced to,

$$\tilde{C}_i^l = C_i^l \frac{V^l}{V^l + V^s} \quad (2.7.8)$$

For the liquid phase, and the condition (2.7.6) it is reduced to,

$$V^l \gg V^s \quad (2.7.9)$$

This simply signifies that the system is very dilute and that the volume of the liquid phase is nearly equal to the working volume. Just as above, the volume of the solid phase is not generally known. Let us introduce phase density that is easily accessible. The volume of the solid phase can be expressed through the relationship of the total mass of the solid and its density,

$$V^s = \frac{M^s}{\delta_s} \quad (2.7.10)$$

By multiplying the right-hand side by  $V_T$ , and by using the definition of phase density, the following is found, (cf. (2.3.14))

$$V^s = X^s \frac{V_T}{\delta_s} \quad (2.7.11)$$

By introducing this value into (2.7.8), the following is obtained,

$$\tilde{C}_i^l = C_i^l \frac{V^l}{V^l + X^s \frac{V_T}{\delta_s}} \quad (2.7.12)$$

Taking into account that

$$V^l = V_T - V^s \quad (2.7.13)$$

Equation (2.7.8) can then be written as

$$\tilde{C}_i^l = C_i^l \frac{V_T - V^s}{V_T} \quad (2.7.14)$$

and by using (2.7.11) and by simplifying, it is found that

$$\tilde{C}_i^l = C_i^l (1 - X^s / \delta_s) \quad (2.7.15)$$

## 2.7.2 Numerical Example

Say, there is a reactor comprising a cell phase of phase density (biomass) 3 gDW/L (DW = dry weight). With a water content of 70 %, a wet biomass of 10 gWW/L (WW = wet weight) is obtained. The working volume of the reactor is 250 mL. By using (2.7.11), it is found that the volume of the cell phase is

$$V^c = \frac{10}{1000 \times 4} = 2.5 \times 10^{-3} \text{ L.}$$

using a cell density of 1000 g/l as above (cf. Sect. 2.2).

According to (2.7.13) the volume of the liquid phase is then

$$V^l = 0.25 - 2.5 \times 10^{-3} = 0.2475 \text{ L}$$

about 100 times greater than the cell volume. Then it can be conceded that (2.7.7) is true and the *R*- or *E*-concentrations can be used interchangeably for the compound *i*. In other terms, the biomass is sufficiently small to assimilate the experimental concentration of *i* (*R*-concentration) in its pseudohomogenous concentration.

### 2.7.3 Critical Biomass

Let us try to estimate the limit of the biomass that can make possible this approximation. Let us represent the factor that intervenes in (2.7.15) by  $f_v$ :

$$f_v = (1 - X^c / \delta_c) \quad (2.7.16)$$

The following table gives values of  $f_v$  for different values of biomass (always supposing that the specific cell mass equals 1000 g/L and that the water content is 70 %) (Table 2.1).

In a system that can be considered as essentially made up of a liquid and a solid phase, the error is less or equal to 3 % if the biomass is less or equal to 10 gDW/L. This error can be considered as acceptable compared with experimental error over biomass measurement. Following this, it is considered that the correction is not significant for systems in which the biomass does not exceed 10 gDW/L. (So a value is obtained that is clearly lower than that conceded by Monbouquette (1987) who adopts from 20 to 25 gDW/L.) The critical biomass should be considered at present as a relatively arbitrary quantity, essentially depending on experimental measurement errors. However, it is not absurd to ask if there is another criteria (kinetic, for example) that could make this value more objective. (Such a study is not known to have been done.) For high or a very high density systems phase

**Table 2.1** Variation in the correction factor between *R*- and *E*-concentrations according to biomass

$X^c$ gDW/L	$X^c$ gWW/L	$f_v$	$(1 - f_v) \cdot 100$ (%)
0	0.00	1.00	0
3	10.00	0.99	1
10	33.33	0.97	3
30	100.00	0.90	10
150	500.00	0.50	50
300	1000.00	0.00	100

density, it is estimated that the correction becomes useful, for mathematical modeling of the process, in particular. (Most often, this modeling serves for the process control.) We insist on the fact that, in the absence of a critical biomass objectification, one must keep in mind the aim of the measure and the value of experimental error (the experimental error can be very high in an industrial process), before fixing an inescapable value for the critical biomass.

### 2.7.4 *The Case of a Gaseous Phase*

Dispersed systems that have a gaseous phase constitute a special case. In fact, this can only be maintained in an open system according to this phase. As soon as aeration (for example) ceases, the gaseous phase disappears and the system is simplified. During a sampling, for example, the degassing is almost instantaneously done and the amount of dissolved gas gives rise to a (generally) negligible volume variation.

During aeration, on the contrary, the working volume can sometimes be considerably modified by the presence of bubbles from the gaseous phase. It is therefore necessary to be careful to be careful when defining the working volume and this is not necessarily a straightforward operation. In fact, the working volume can be defined in two ways; one consists of defining the working volume without aeration ( $V_T$  (NG)) and the other with aeration ( $V_T$  (G)). Depending on the applications, the two methods can be useful, but needless to say, only the working volume with aeration corresponds to the real situation. It is easy to note that relationship (2.7.6) can be considerably influenced by the gaseous phase, even if the solid phase is small compared to the liquid phase. If the working volume is defined with the gaseous phase, approximations defined in the preceding paragraph remain valid. Otherwise, modifications must be made. Unfortunately, in the literature, the exact characterization of the used working volume is not generally well defined. Below, when the data from the literature is used, it is supposed that the working volume given is that without the gaseous phase and the approximation of 10 gDW/L will be kept as the critical biomass before applying corrections. However, it is clear that our hypothesis does not in any way exclude the risk of some bias. Fortunately, the considered examples have biomass well below 10 gDW/L, which reduces the error range.

## 2.8 **The Variation of the Internal Composition of a Microorganism with the Growth Rate Is a Consequence of the Mass Conservation Law**

It has long been known, and in a very general manner, the composition and size of a microorganism depends upon its growth rate. This dramatically applies to bacteria. We quote: “*The growth rate that a particular medium supports, not its specific*



composition, determines the *PHYSIOLOGICAL STATE* (cell size and macromolecular composition) for cells growing in it. For example, the physiological state of cells is the same in different media or even in continuous culture if the cultures are all growing at the same rates.” (Neidhardt et al. 1994). (In a continuous system, this also applies to the growth of crystals, for example Villermaux 1982.) Here we show that the PDS formalism can easily enlighten these facts. A more detailed demonstration can be found in Thierie (1997).

The chemostat is discussed in detail in the following chapters, however, to have control over the growth rate, we still use this bioreactor as an example (see Sect. 2.6).

Consider the general mass balance Eq. (2.4.26) in the form

$$\frac{d\tilde{C}_i^p}{dt} = \frac{1}{V_T} (\tilde{F}_i^E - \tilde{F}_i^S) + q_i^p X^p + \tilde{C}_i^p \left( \frac{d \ln N_T^p}{dt} - \frac{d \ln V_T}{dt} \right) \quad (2.8.1)$$

This equation is valid for any compound  $i$ , in each phase  $p$ .

Let us apply this general relationship to a strictly intracellular compound  $i$ , in a constant volume chemostat. Assume also that there are no cells inflowing within the chemostat. When all these conditions are satisfied, in the steady state (2.8.1) is reduced to:

$$0 = \frac{-\tilde{F}_i^S}{V_T} + q_i^c X^c \quad (2.8.2)$$

Taking (2.4.25) into account and rearranging:

$$0 = r_i^c \frac{X^c}{\delta_c} - D\tilde{C}_i^c \quad (2.8.3)$$

Using the definition of  $E$ -concentrations to write mean concentrations, it comes after reordering that

$$0 = (r_i^c - DC_i^c) \frac{X^c}{\delta_c} \quad (2.8.4)$$

from which we obtain that finally, the steady state condition is

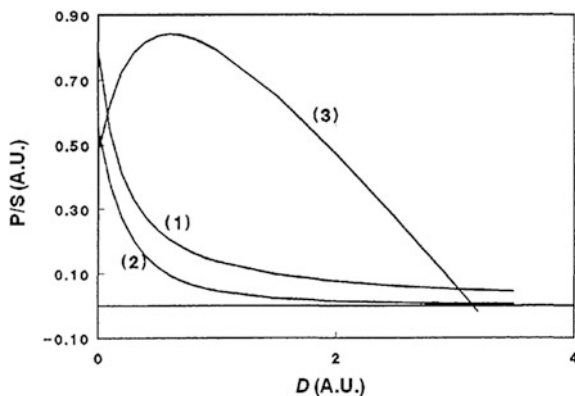
$$C_i^c = \frac{r_i}{D} \quad (2.8.5)$$

This relationship shows that the mean composition of a strictly intracellular compound  $i$  depends on the growth rate ( $D$ ), and this irrespective of the intracellular transformation rates ( $r_i$ ) of this compound. (Obviously, transformation rates can be very complex and depend on many factors. Equation (2.8.5) therefore does not imply that the internal concentration inversely varies with  $D$ .)

Mass balance, which is an implicit relationship (no “assumption” other than matter conservation) therefore provides a nontrivial and extremely general result (2.8.5).

The result seems incontestable and the steady condition (2.8.5) is a necessary condition. But is this condition sufficient? In other words, does the steady intracellular concentration condition (2.8.5) adequately fulfill both mass balance requirements and cellular effectiveness simultaneously? The question is not obvious, but we are tempted to believe that this is not the case. We know that the steady cellular growth in a chemostat is only possible in a bounded range of dilution rates. Beyond a critical value, the cellular growth fails to counterbalance the hydraulic output and the system undergoes a washout (all the cells are “washed out” of the vessel). It is possible to interpret this well-known phenomenon in terms of a conflict between the metabolic requirements for growth and the necessity to satisfy the concentration values imposed by the law of conservation of mass. The Fig. 2.10 illustrates this idea.

The curves represent the steady states of a chemostat as a function of  $D$ , for three different possible metabolic reactions (see Figure caption). For two of them (curves (1) and (2)), the ratio  $P/S$  (Product/Substrate (or reactant)) decreases asymptotically to zero but vanishes only as  $D$  tends to infinity. For finite values of  $D$ , the concentration of the product may become very small but the metabolite is always produced. Theoretically, there is no reason to expect an event to some particular value of  $D$ . On the contrary, the third mechanism (curve (3)) leads to a complete exhaustion of the metabolite at a precise value of  $D$  (around  $3.2 \text{ t}^{-1}$  in our example).



**Fig. 2.10** Steady states of a chemostat as function of dilution rate,  $D$ , for different intracellular kinetics.  $S$  is a substrate (or reactant) leading to the production of  $P$  (product). The  $P/S$  ratio is plotted versus  $D$ . ([A.U.]: arbitrary units) Curve 1: Monomolecular reaction:  $S \xrightarrow{k} P$ . Curve 2: Bimolecular reaction:  $S + S \xrightarrow{k} P$ . Curve 3: Michaelis–Menten mechanism:  $S + E \xrightleftharpoons[k^-]{k^+} (ES) \xrightarrow{k} P + E$

If the metabolite is indispensable for cell growth or viability, a critical dilution rate exists beyond which the washout occurs. Experimental protocols like those depicted in Fig. 2.2 ( $P/S = f(D)$ ) may lead to interesting information about the kinetics of two metabolites suspected to derive from each other in a more or less direct fashion. Systematic inspection of such  $P/S$  ratios as a function of dilution rate reveals specific behaviors and may be used to highlight particular links and specific physiological properties.

Without any interpretation, the concentration change due to the mass balance obscures, in some sense, the role that a metabolite may play in the growth process. Some metabolites (or organelles, like ribosomes) are supposed to play a particular and important role in the mechanism of cell growth. The correlative change of those compounds with growth rate is interpreted as governing the cellular growth. We know now that one must be very circumspect in doing so, because a change in concentration with growth may be “active,” that is to say, necessary to permit this particular rate, or “passive,” being a consequence of this specific rate and simply obeying the mass conservation law.

To conclude, by mean of a pseudohomogenous description of a polyphasic system, we have analyzed the consequences of the growth rate on the concentration of a strictly intracellular compound of a cell cultivated in a chemostat. Without any explicit kinetic representation, the results are kept at a qualitative level. We have chosen this example, because the effects are well known in microbiology and bioengineering and illustrate the relevance of our approach. The method, even at a qualitative level, permits to explore the influence of other parameters than the dilution rate (like external kinetics, phase number, and so on), perhaps leading to unexpected predictions and interesting challenges for experimenters. By use of explicit kinetics, our formalism must be able to provide quantitative results, allowing to conceive new perspective in modeling and different optimization and monitoring concepts.

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