1

Bioreactors

1.1. Introduction

1.1.1. *What is a bioreactor?*

A bioreactor is an enclosure containing a nutrient medium consisting of a cocktail of various molecules – referred to as "substrates" – upon which one or more populations of microorganisms grow, and as such the set of these microorganisms is called "biomass". Bioreactors are used to perform operations for transforming matter through biological pathways, most often accompanied, but not systematically, by the increase of biomass in the reaction medium. Microbiology teaches us that only soluble substrates – that have created chemical bonds with water molecules – are available for the growth of living cells. Within the context of this book, from a formal point of view, a biological reaction will therefore describe the transformation of elements existing in the medium in soluble form into a solid form, biomass and possibly into a certain number of metabolites and/or gas. However, it may happen that a number of resources are present in solid form. A so-called "hydrolysis" step is necessary to transform this solid substrate into a soluble form assimilable by microorganisms. The conception that claims that a microorganism "grabs" molecules passing nearby is somehow a figment of the imagination. For example, it is accepted in soils that most microorganisms, whether they be mobile or not, excrete enzymes around them and recover the nutrients that reach them by diffusion. It is therefore essential to properly distinguish between the different processes involved before initiating the modeling of phenomena as complex as the degradation of a set of substrates by microorganisms. This is the subject of this chapter that describes the most important processes involved in this type of matter transformation and the systematic approach widely adopted in process engineering to model them. In what follows, we will only cover microbial ecosystems that are implemented in most bioprocesses.

1.1.2. *Classification of biological reactors*

In process engineering, bioreactors are first classified according to their mode of operation, in other words the way in which they are supplied with matter, and depending on whether microorganisms are free in the medium (so-called "planktonic" organisms) or fixed on a support; the latter could itself be fixed or mobile.

As a result, it is possible to distinguish continuously-fed systems, systems whose supply is semi-continuous and those operating in closed mode. In continuous reactors, the reaction volume remains constant, in- and outflow rates being identical. It is the most commonly used operating mode in industries aiming to process a large amount of material arriving continuously, as it is the case, for example, in the treatment of water by biological means. We will often refer to this mode in this chapter insofar as this is one of the most significant industries in terms of quantities of processed materials. Semi-continuous (or *fedbatch*) reactors are systems whose inflow rate is not zero but whose outflow rate is zero. In such a system, the reaction volume is thus increasing over time from a minimal to a maximal value. This type of system is particularly suitable for the production of biomass as the amount of substrate can be supplied according to the specific needs of microorganisms. It is also used when the risk of inhibition due to the substrate accumulation or a metabolic intermediary in the medium is present. Depending on the physiological state of microorganisms, it is then possible to decrease, or on the contrary, to increase the amount of resource fed into the reactor. Finally, batch mode – or reactor – designates a closed system in the sense where there is neither supply nor withdrawal of the system: substrates (the different nutrients necessary for the growth of microorganisms) as well as the inoculum (biomass) are introduced at the initial time. Therefore, the reaction volume of the system is constant over time (if possible liquid-gas exchanges are neglected) and the reaction takes place up to the moment when it is measured (or considered) that it has completed. This operating mode is widely used in agri-food, pharmaceutical and chemical industries, notably for the production of molecules with high-added value, and more generally in cultures in which the risk of contamination through the feed is high.

Since biomass is the catalyst for reaction, the effectiveness of a biological system will be all the more significant when the substrate necessary for its growth is in an appropriate form (this is referred to as biodegradability) and accessible (so-called accessibility). The homogeneity of the medium as well as biomass and resource densities will consequently play essential roles in the operation of these systems. In order to maximize the concentration of the existing biomass – but mainly to facilitate the separation of the biomass from the residual reaction medium (in other words, to facilitate the separation of the liquid and solid phases of the medium) – it is possible to resort to using a support upon which biomass will tend to settle in the

form of "biofilms"[1]. In laboratories, even today, many engineers are testing the effectiveness of all kinds of fixed or mobile supports and are studying the properties of associated processes. In fact, it is essentially based on these considerations – relating to the feeding modes of reactors and to the manner in which biomass is retained within the system – that different technologies of reactors have been proposed. Finally, the last major element for the classification of bioprocesses is linked to the same biological processes that condition bacterial growth. It designates the set of conditions that must prevail in the medium to enable the growth of microorganisms (this is often referred to as "environmental conditions"). They are essentially ecosystem-dependent. In the next section, we review a certain number of concepts that are necessary to understand the formalization of the model of the chemostat, which we will next present in several ways in the book.

1.1.3. *A brief reminder of microbiology*

To grow and multiply, a (micro-) organism needs a multitude of elements, including some at trace level. A natural manner to classify organisms is to refer to the mechanisms that they implement to capture the matter necessary for their growth and to produce their energy. When we concentrate on the major factors influencing growth, a source carbon and an energy source are in effect essentially needed. Carbon is found in two basic forms in nature: organic or inorganic. For simplicity, assume here that organic carbon is the carbon produced by living entities, inorganic carbon designating the CO_2 in its different chemical forms (carbonic acid, bicarbonate, dissolved CO_2; we will later on return to these elements to talk about the mutual influence of biological reactions and chemical balance of the medium, which are fundamental factors affecting the growth of microorganisms).

There are essentially two sources of energy: light and chemical energy. Organisms that extract their energy from light are called phototrophs, those who take it from chemistry being called chemotrophs. Regarding sources of carbon, organisms being able to utilize organic matter are called heterotrophs, those using CO_2 are known as autotrophic. By combining the carbon and the energy source being utilized, four major classes of microorganisms can then be defined:

– chemoautotrophs utilize CO_2 as a carbon source and derive their energy from the consumption of inorganic substrates;

– chemoheterotrophs utilize organic matter as a source of energy and carbon;

– photoautotrophs utilize CO_2 as a carbon source and derive their energy from light;

1 Barring a few exceptions, it may be helpful to recall here that the natural form of microbes in the medium involves that they precisely structure themselves into biofilms and flocs, the planktonic state being specific to certain species.

– photoheterotrophs utilize organic material as source of carbon and light as energy source;

In environmental biotechnology, chemoheterotroph bacteria have been particularly studied because they degrade organic matter and are not constrained by light, which is difficult to evenly diffuse inside reactors of large volumes comprising high cell densities. The metabolism of these organisms enables energy to be produced and the final products of the degradation are CO_2 and water. The other bacteria widely studied in the environmental field are chemoautotrophs because they are involved in the nitrogen cycle. They derive their energy from the presence in the medium of molecules such as ammoniacal nitrogen or nitrite and their source of carbon is CO_2.

Why have we presented these various notions in a book dedicated to the chemostat? Based on the previous few examples, we will show in the next section that the apparent complexity of the functioning of microbial ecosystems can be formalized in a simple and natural manner at the populations level, regardless of the type of metabolism implemented by the microorganisms.

1.2. Modeling of biological reactions

1.2.1. *Regarding the state variables of the model*

As indicated at the beginning of this chapter, a chemostat consists of an enclosure in which resources are provided by means of a feed and from which the reaction medium is withdrawn at an outlet (Figure 1.1). Assuming that all the elements necessary to the growth of the microorganism that will develop inside the reactor are present in excess at all times, the velocity at which it could develop would be limited only by its own ability to "ingest" substrates and at the velocity with which the medium is renewed. Assuming that its velocity of growth at time t depends on its own density (which means that with a biomass concentration twice as high, the velocity would be twice as significant), its growth would be exponential until the resource or space start lacking. In practice, this situation can only be observed during the transitional phase and, if it is not space, a resource at least, said to be "essential", always becomes scarce and limit growth (hence its name of "limiting" resource or substrate). In microbiology, this is most often the carbon or nitrogen source but, assuming they impact on the growth velocity in a similar manner to a substrate, it can equally concern light or the concentration of oxygen, that is to say, the source of energy. To achieve modeling of the chemostat, we will assume that all the necessary conditions for the growth of a microorganism are gathered except for one. In other words, with the exception of an essential substrate, it is assumed that all the nutrients needed for growth are present in excess.

It should be observed that the terms s and x both commonly denote substrate and biomass as well as their respective concentrations (thus in mass per unit volume). In

this respect, let us clarify for a moment what "biomass" designates here. A microorganism, such as any living organism, is mainly composed of water. Therefore, how can a concentration of microbes within a biological reactor be characterized? Besides the available measures to which we will return to very quickly, we can address a quite fundamental question – and still subject to debate among scholars – namely the most appropriate terminology to define a set of microbial individuals that perform the same function within an ecosystem. Historically, the term "pure culture" is used to designate microorganism growth within a reactor that has been inoculated with a colony originating from a unique microbial cell. The reactor is then designed such that the ecosystem cannot be contaminated by another microorganism. In this case, this is known as "microbial strain". After numerous subcultures, some evolution will, however, be noticed in the genome of daughter cells. From the moment these differences become identifiable, but remain minor, it is then referred to as "species", a term borrowed from the classification of macroorganisms. Despite being correctly defined in the context of sexual reproduction, it has long been used in the microbial world even if modern methods of molecular biology, which have particularly highlighted that "two clones were never completely so", appearing to raise once again the issue of the existence and relevance of the notion of species in microbes (see [WAD 16]).

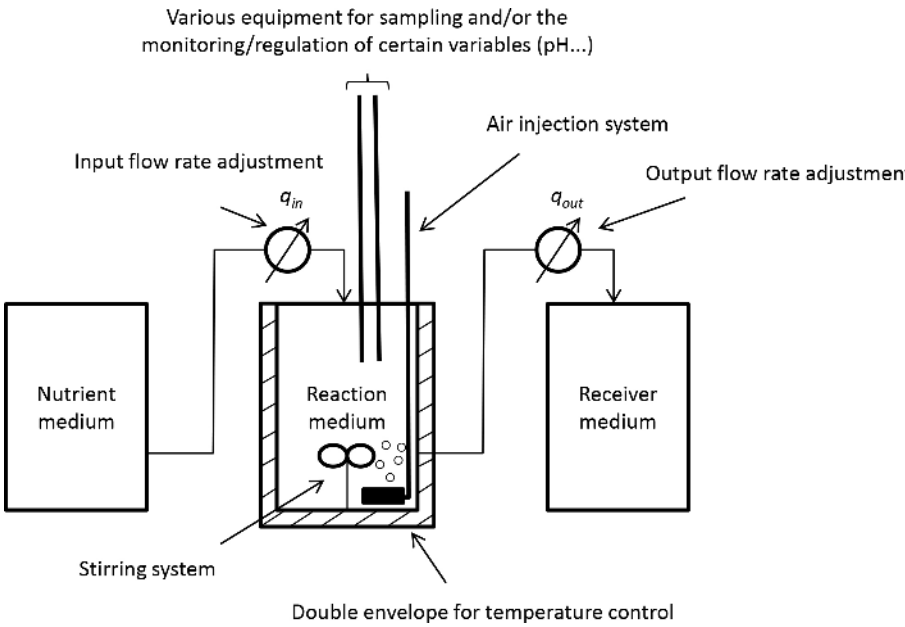

Figure 1.1. *Schematic representation of a chemostat*

The somewhat opposite concept to “pure culture” is that of “mixed culture” where existing microbes can be of different natures and perform very different functions within the ecosystem. In reality, this is of course the case of any natural ecosystem in which a large number of microorganisms live with very different characteristics. It is interesting to specify here for neophytes that a larger number of differences might be identified between the genomes of two microorganisms than between a human and a goldfish. The purpose of this book is not to address these expert issues, and we will merely define the terms, used interchangeably in this book, strain, species or even biomass (although this last term will preferably be used to designate the set of the active population): namely a set of microbes performing an identical function with respect to one or more limiting resources in a growth medium. Nonetheless, it is important to be aware that this relates here to a crucial point of debates centered on the chemostat and its modeling in particular. As a matter of fact, we will further discuss in this book questions related to diversity and coexistence using as a definition of species the one we just have given. Therefore, as we have just clarified, the function of a species is established once and for all in our models.

In fact, numerous ecologists remain convinced that modeling works, when they are applied to issues notably concerning diversity and do not raise much interest as long as the notion of evolution (the way in which the functional capabilities of the species change) is not taken into account. Without getting into very detailed arguments, we will first address these reservations that the chemostat – and its model – have initially been proposed by biologists based on technical developments and experimental difficulties: Monod and Szilard were indeed biologists. In fact, the chemostat is not – not only in any case – a figment of the imagination of mathematicians. Then, despite the fact that the models that we use, and which are to be seen here as models “to think”, are necessarily very simplified visions, or even naive, of reality, it does not lessen their ability to provide a means to formalize knowledge. Their study allows the testing of assumptions, especially concerning the mechanisms involved in the reaction medium, and as a result they constitute a terrific communication tool, for exchanges and reflections between researchers whether within but even more outside of their disciplinary fields. It is too often forgotten that a model is primarily a tool for the formalization of knowledge and – not only – a tool for prediction as a means to “stick to data”.

This issue of the characterization of biomass immediately recalls the technologies available for characterizing the populations of microorganisms. From the outset, these techniques raise a number of issues that sometimes appear very complicated to answer. For example, if a quantification technique requires a significant volume of samples (compared to the volume of the reactor), it could be asked to what extent this sampling will disrupt the operation of the system. What can be done to quantify the existing biomass? Let us immediately clarify that we exclude from our remarks the case of the characterization of biomass structured in flocs and in biofilm that raise technical questions which largely fall beyond the issues addressed in this book. When they are

free in the medium (we recall that this is referred to as "planktonic" organisms), we know how to count cells, to characterize a structuring according to the population size or even to weigh matter by means of various techniques. It is also possible to resort to molecular biology in order to quantify the abundances of genes. However, since microorganisms reproduce through cell division, they are not all in the same physiological state at a given time. In addition, it is particularly complicated, not to say impossible, except in very particular study situations, to know which ones are active – in the sense where they participate in the observed consumption of substrate – and which are not. It should be noted that the very notion of dead microorganism must be specified since a cell may very well be undivided but inactive and that a lysed cell can release active enzymes in the medium.

These remarks have led scientists to quickly ask themselves the question about knowing what was the most appropriate characterization for measuring biomass. In the majority of cases, it implies that the effects of mortality have thus to be ignored based on the fact that, considering very large populations, their consequences are negligible on the manner in which biomass as a whole behaves with regard to a limiting resource within a continuously operating reactor. Another way to state it is that in certain situations, biomass mortality will be neglected compared to the momentum generated by the renewal of the medium.

Getting back to the measures available to quantify a concentration of microorganisms, we may make use of the concentration of "volatile matter". Considering now the technical details, the concentration of volatile matter in the medium is obtained in the following way: first, the liquid and solid phases of the sample are separated (for instance through centrifugation). We then proceed to a first weighing of the pellet (solid matter): in this way, the "concentration of solids or solids in suspension" is obtained. Assuming that there is no organic matter in solid form in the medium or in the feed, it should be noted that this concentration may be a first characterization of x. Nevertheless, this estimate can be refined by excluding from this quantity the set of minerals, assimilated to resources rather than to biomass: to this end, the sample is oxidized (through combustion) such that all of the organic matter is oxidized into gas composed of CO_2 and H_2O and the remaining matter is weighed. The difference between this last measure and solid matter gives us the volatile matters often assimilated, in biotechnology, to biomass. The fact that only part of the matter is active and the rest is the reflection of dead cells is usually overlooked due to the remarks formulated earlier. An alternative very often used if the medium is neither colored nor too loaded with suspended matters (with solids) is to measure the turbidity of the medium (its "blurred" character). This is an optical measure widely utilized in biotechnology because it is very simple to implement, even continuously with "turbidimeters" directly immersed in the medium. Nonetheless, any wastewater treatment applications, excessively loaded with matter in suspension, and more generally all those in which the environment influences the measure independently of the biomass concentration (a substrate itself can disturb the

medium) cannot use it. It is also possible to adopt a formalism in which biomass and substrates are quantified in the same unit. This is how water treatment engineers proceed for whom biomass and substrates are characterized based on using the chemical oxygen demand or COD. The COD is a standard measure of pollution as well as a means for easily establishing overall matter balances. Furthermore, the incoming COD is subdivided into different compartments, all quantified *via* the same measure. Finally, it is possible to use a number of means for indirect measurement (conductivity, infrared, photometry, etc.), keeping in mind, however, that all these devices can be very useful for the systematic online monitoring of biomass. However, they have to be calibrated and therefore it is necessary to resort to a standardized concentration unit, which brings us back to the original question. First, it can be retained therefrom that adopting a unit for biomass is a challenge in itself and depends on the means available, not only human resources and equipment but also financial.

Fortunately, we are equipped with some background on the usage of these different techniques whose advantages and limitations are now quite clear. The purpose of our book is not to conform models to reality but to study their properties by means of mathematical analysis. Moreover, the key point to remember is that, later in this book, appropriate units will always be used such that up to a performance coefficient, what we claim remains valid for what has been defined as being s and/or x.

1.2.2. *Biological processes and reaction scheme*

The reaction scheme describes the desired processes to be formalized during a given biological reaction. It is inspired by the way in which reactions in chemistry are represented. A biological reaction can be seen as the transformation of matter catalyzed by the presence of a microorganism. A very intuitive manner to represent the growth of a microorganism x on a substrate s is to use the formalism of chemistry for an irreversible reaction, namely:

$$s \rightarrow x \qquad [1.1]$$

that schematizes a transfer of matter from s toward/into x.

Here, the notations x and s have no unit. They are used to describe a qualitative transfer of matter, in some way a sense of reaction. In particular, there is no notion of balancing the reaction: we merely define a scheme where a matter compartment in a given form (here s) is converted into another compartment (x). This is the reaction scheme. This information can be completed by the fact that, for example, this reaction is accompanied by the production of CO_2. In this case, a term will be added to the right:

$$s \quad \rightarrow \quad x + CO_2 \qquad [1.2]$$

To be a little more specific, we can still get closer to the formalism of chemistry by indicating reaction yields and kinetics. However, we should bear in mind, once again, that yields are not originating from balancing the reaction in the sense of chemistry, but from the knowledge made available through *ad hoc* trials by end users: with regard to biomass growth, we would have previously established what is meant by "biomass" and that, according to this definition, "such amount of substrate is necessary to produce such amount of biomass". We then obtain the following scheme:

$$k_1 s \quad \stackrel{\rho(.)}{\rightarrow} \quad x + k_2 CO_2 \qquad [1.3]$$

which reads "the growth of a biomass unit is achieved from k_1 substrate units and is accompanied by the production of k_2 units of CO_2 at velocity (or *rate*) $\rho(.)$" (in mass per volume and per unit of time).

Let us for a moment consider the notion of yield. Assume that an organism grows in aerobiosis, on glucose (with chemical formula $C_6H_{12}O_6$) – it is thus a chemoheterotroph microorganism – and that glucose is in limited quantity. If we utilize elemental composition data from microorganisms "in the main elements that constitute them on average" (for example here in carbon, hydrogen and oxygen), it will be then possible to assimilate the biomass to a chemical formula and to use this time the formalisms of chemistry to calculate the values of these yield coefficients. Therefore, model average compositions of microorganisms can be found in the literature (see [TRU 09]). The reader should also notice that, in the formalism of the previous example, the yields can be normalized by defining k_1 or k_2 as equal to the unit, such that only a single yield parameter is considered. This parameter will be denoted by $\frac{1}{Y} = \frac{k_1}{k_2}$ (or its converse depending on whether we are considering the "consumed substrate or CO_2 produced"), which will limit the number of parameters of the model. Moreover, this is the notation that we will use in section 1.3.1. At this stage, there is no need to clarify what exactly $\rho(.)$ is and we will merely keep in mind that it is homogeneous to a velocity.

In the most elementary form previously used, the reaction scheme is capable of specifying that only a single biological process – here growth – is involved. However, according to the formalization being considered, the processes to be modeled can be multiple: biomass can, for example, be subject to "mortality". In this case, the previous scheme has to be completed by a second equation:

$$k_d x \quad \stackrel{x}{\rightarrow} \quad x_d \qquad [1.4]$$

which describes the fact that the "active biomass" compartment feeds a "dead biomass" compartment (denoted here by x_d) with a rate k_d.

In addition to mortality, the processes of interest in microbiology are numerous. We give hereafter some examples of processes which have been considered in the literature dedicated to modeling in microbiology:

– maintenance is the use of part of the resources for the supply of energy to biomass. It is usually modeled by adding a negative term (also known as "well") in the equation of the resource without modification of the dynamic equation of biomass, which correctly describes a disappearance of substrate without microbial growth;

– although different in terms of process, the definition of maintenance is sometimes confused with a mechanism called "endogenous respiration" that describes a situation in which the biomass itself becomes its own substrate. In other words, it concerns a process for recycling the matter based on biomass mortality. To model it, a negative mortality term can be added to the equation of biomass associated with a positive term in the dynamics of the substrate. Since there is necessarily less consumable substrate that appears than biomass that disappears in a given unit, this term is sometimes not added to the biomass due to the fact that, by extension, mortality and maintenance are then modeled by the same modifications to the equations. This situation is an excellent example of the contribution of modeling to the formalization of microbial dynamics: if general definitions are considered excessively and not formalized in the form of equations, it may result that some processes of different nature, however, appear as identical. From the moment an equation is stated, it is important to properly define the assumptions that allow us to write it. If the maintenance describes a substrate consumption without bacterial growth, then only the introduction of a negative term in the substrate is consistent. If maintenance describes a way for biomass to maintain itself in the absence of substrate, then it concerns the recycling process of nutrients modeled by the simultaneous addition of a mortality term in the dynamics of biomass and a source term in the dynamics of the substrate;

– flocculation is the structuring of biomass into flocs. As noted above, microorganisms naturally tend to agglomerate in biofilms and flocs. A more realistic description than the one that only considers planktonic microorganisms, thus involves adding a compartment of structured microorganisms to the model, for example in flocs;

– as mentioned in the introduction of this chapter, it may happen that the limiting resource in the feed may not be accessible to microorganisms, for example because it is found in solid form. In this case, it is necessary to add a hydrolysis step describing the manner how – velocity and yield depending on the conditions of the medium – this matter compartment feeds a biodegradable and accessible substrate compartment.

Keeping these general points in mind, let us now address the modeling of a simple biological reaction.

1.2.3. *Chemostat equations*

Prior to establishing the chemostat equations as such, we specify a few conventions for the notations that we will use throughout the book. To do this, it is helpful to recall here a common notion of automatic control, namely that of a system input. If we define the system of the chemostat as comprising an enclosure as well as two supply and withdrawal devices notably, including pumps, that allow the user to set inflow and outflow rates, it then becomes possible to identify variables allowing us to interact with the system. These variables are precisely the two feed and effluent flow rates and eventually the concentration of feed, of which we can picture that an *ad hoc* device allows the user to fix it to a given value. The dynamics of the volume of the reactor and the biomass and substrate concentrations inside the chemostat (constituting the state of the system) are then governed by non-independent dynamic equations insofar as variations of the input variables will lead to changes in the system state variables. By convention, for these input variables (here, the two feed and outflow rates in addition to the concentration of substrate in the feed), we will use terms in uppercase for constant values and in lowercase when these variables are likely to vary over time.

To establish the equations of the chemostat, we use here the usual formality of process engineering by directly applying a mass balance to s and x. Consider an enclosure of volume v equipped with an inlet – the supply of the reactor referred to above – and an outlet through which the reaction mixture can be withdrawn. In order to be as formal as possible, we assume that this facility is equipped with all the necessary control devices so that the mixture is homogeneous. In addition, it is also assumed that from a reactive point of view, environmental conditions (in particular temperature and pH) are constant (in the sense that they are not the reason for the variations observed in concentrations of interest).

From now on, s_{in} (or S_{in}) should denote the concentration of s in the feed, q_{in} and q_{out} (or Q_{in} and Q_{out}) the inflow and outflow rates (in volume per unit of time) and $Y(\cdot)$ the yield of the conversion of substrate into biomass (in mass of substrate consumed per mass of biomass produced). In the great majority of articles available in the literature, yields are constants. However, to explain oscillations that appear in some experiments, some authors have proposed regulatory mechanisms resulting in considering yields as functions of substrate and/or biomass concentration. In most cases, we will therefore denote yields by $Y(\cdot)$ in this chapter. The reader should also notice that we have used coefficients k_i in reaction schemes to not show terms in $\frac{1}{Y}$. According to each one's modeling cultures, conversion yields are thus differently denoted and are thus homogeneous either to a mass of substrate consumed per mass of biomass produced, or conversely, that is to say to a mass of biomass produced per mass of consumed substrate. The latter are by definition non-zero, the notation does not matter in the present case and in the following we will opt for the notation in use in the field of wastewater treatment engineering, namely a term in $\frac{1}{Y}$ in the dynamics of s. Now, consider the simpler reaction scheme [1.3] with $k_1 = \frac{1}{Y}$ and focusing on the

dynamics of s and x. Let us achieve a mass balance according to which, for a period of time dt, the variation in the mass of an element (here s and x) is the result between the mass of that element that has been brought into the system added to the produced mass of this element minus the consumed quantity minus the extracted quantity. If this principle is applied to the biomass and to the substrate in the reaction volume v of the reactor, the following equations are obtained:

$$\begin{cases} \dfrac{dv}{dt} &= q_{in} - q_{out} \\ \dfrac{d(sv)}{dt} &= q_{in}s_{in} - q_{out}s - \frac{\rho(.)}{Y(\cdot)}v \\ \dfrac{d(xv)}{dt} &= \rho(.)v - q_{out}x \end{cases} \quad [1.5]$$

Without loss of generality, we will of course be cautious to only consider rates q_{in} and q_{out} that maintain v positive. Noting that $\frac{d(uv)}{dt} = u\frac{dv}{dt} + v\frac{du}{dt}$, we can express the dynamics of the concentration of x and s that are more easily manipulated in chemistry than masses inasmuch as it is desirable to reason regardless of the volume of the reactors. It thus yields:

$$\begin{cases} \dfrac{dv}{dt} &= q_{in} - q_{out} \\ v\dfrac{ds}{dt} &= q_{in}s_{in} - q_{in}s - \frac{\rho(.)}{Y(\cdot)}v \\ v\dfrac{dx}{dt} &= \rho(.)v - q_{in}x \end{cases} \quad [1.6]$$

or still:

$$\begin{cases} \dfrac{dv}{dt} &= q_{in} - q_{out} \\ \dfrac{ds}{dt} &= \frac{q_{in}}{v}(s_{in} - s) - \frac{\rho(.)}{Y(\cdot)} \\ \dfrac{dx}{dt} &= \rho(.) - \frac{q_{in}}{v}x \end{cases} \quad [1.7]$$

Let us now define $\rho(.) = \mu(.)x$ where μ is called "specific growth velocity" (we will return to this point in the next section). This assumption is reasonable: having defined the biological reaction as being catalyzed by the presence of biomass, it simply guarantees that the growth velocity is zero in the absence of biomass.

This model is a means to obtain the equations of dynamics of s and x for all three modes of operation of interest that we have outlined in section 1.1.2, namely:

– the closed mode (or *batch*) where $q_{in} = q_{out} = Q_{in} = Q_{out} = 0$, $v(0) = V$, $x(0) = X_0$ and $s(0) = S_0$:

$$\left\{ \begin{array}{rcl} \dfrac{ds}{dt} & = & -\frac{\mu(.)}{Y(\cdot)}x \\ \dfrac{dx}{dt} & = & \mu(.)x \end{array} \right. \quad [1.8]$$

– the semi-continuous (or *fedbatch* mode) where $q_{out} = Q_{out} = 0$, $v(0) = V_0$, $x(0) = X_0$ and $s(0) = S_0$:

$$\left\{ \begin{array}{rcl} \dfrac{dv}{dt} & = & q_{in} \\ \dfrac{ds}{dt} & = & \frac{q_{in}}{v}(s_{in} - s) - \frac{\mu(.)}{Y(\cdot)}x \\ \dfrac{dx}{dt} & = & \mu(.)x - \frac{q_{in}}{v}x \end{array} \right. \quad [1.9]$$

– the continuous mode where $q_{in} = q_{out} \neq 0$, $v(0) = v(t) = V$, $x(0) = X_0$ and $s(0) = S_0$. By denoting $d = \frac{q_{in}}{V}$, which is called the "dilution rate", it yields that:

$$\left\{ \begin{array}{rcl} \dfrac{ds}{dt} & = & d(s_{in} - s) - \frac{\mu(.)}{Y(\cdot)}x \\ \dfrac{dx}{dt} & = & (\mu(.) - d)x \end{array} \right. \quad [1.10]$$

In the form of the system [1.10], we recognize in each of the equations a term related to hydrodynamics – here, transportation terms $-dx$ and $d(s_{in} - s)$, respectively – and a reaction term: $\mu(.)x$ and $-\frac{\mu(.)}{Y(\cdot)}x$, respectively. This model is called the "chemostat model" and it is therefore thereto and to a number of its extensions that we dedicate this book. According to the forms that μ and Y will take, we will see that the dynamics of this system is particularly rich. Please note that we will essentially focus on the study of equations [1.10] in which the substrate feed concentration and the feed rate are fixed ($s_{in} = S_{in}$ and $d = D$).

A first extension of [1.10] consists of considering that the dilution rate which applies to the "substrate" compartment is not the same as the dilution rate of the "biomass" compartment. This modeling assumption is useful for accounting for the presence of a biomass retention device: a fraction of this latter remaining confined

inside the reactor, the term $-d$ in the dynamics of x of equation [1.10] becomes $-\alpha d$. We will see that this modification causes major complications in the qualitative analysis of the system because it prevents using a common change of variable $z = Ys + x$ (which accounts for the conservation of matter inside the system: at steady-state, part of the available resource s_{in} is transformed into x and the complement is a residual substrate s).

At this stage, one may argue, although we have presented several distinct operating modes, why spend most of our efforts in the analysis of continuous models and not also in addressing *batch* and *fedbatch* systems? In fact, the principal advantages of the continuous operation were at the basis of the device itself, namely our ability, at equilibrium, to "drive" the net growth rate of a microorganism. We will return in more detail to this point in Chapter 2. This reason thus leads us naturally to the question of modeling these growth rates, which we will call hereafter "biological kinetic".

1.2.4. *Biological kinetics*

In the previous sections, we have presented a model describing the dynamics according to which matter is transformed by biological means. However, we have not discussed the way in which the velocity – or kinetics – of the reaction can be modeled. The only assumption that has been made so far is that velocity ρ was written $\rho = \mu(.)x$. If we take the equations of growth in *batch*, we have $\frac{dx}{dt} = \mu(.)x$. By definition, the "specific growth rate" refers to the quantity $\frac{1}{x}\frac{dx}{dt} = \mu(.)$. Monod, in his works on the growth of microorganisms, discovered that the function $\mu()$, with fixed environmental conditions, only depended on the concentration of the limiting substrate. In particular, he introduced the function $\mu(s) = \mu_{max}\frac{s}{s+K_S}$ [MON 50]. This function is zero for $s = 0$ and tends toward μ_{max} when the substrate concentration becomes "large" compared to K_S. In addition, he named K_S the semi-saturation constant, denoting that $\mu(K_S) = \frac{\mu_{max}}{2}$. When $\mu = \mu(s)$, the growth rate is proportional to the population density: moreover, all things being equal to a biomass density twice greater, the concentration of microorganisms grows twice faster. Nonetheless, this expression has given rise to numerous discussions. The first criticism that can be made of this expression is that it does not originate from a law (even if it is sometimes presented and named as such) but from a heuristic approach enabling a two-variable function to replicate data in a satisfactory way. In reality, it is inspired by the Michaelis–Menten expression established in 1913 and that which describes the kinetics of a reaction catalyzed by an enzyme (see [JOH 11]). The latter, even if it is not sufficient to describe complex situations, is based on mechanistic bases since a purely chemical formalism makes it possible to establish it. In the case of microbial growth, the situation is different because it is the product of a very large number of intracellular reactions whose result is observed at the population level. In more complex situations, the use of expressions involving a larger number of parameters is necessary. We will here omit the hundreds of

expressions of the literature that have been proposed since Monod's time (see the appendix of Bastin and Dochain's book that lists many expressions of kinetics proposed in the literature [BAS 90]) and we will merely identify the refinements that followed in microbial kinetics modeling:

– it is essential to note here that in most cases, growth rate basically depends on a large number of parameters. For example, it is absolutely intuitive to consider that temperature and pH will play crucial roles in microbial growth: maintaining our refrigerator at 4° makes it possible to limit the development velocity of microorganisms and as a result to preserve our food longer. In order to concentrate on the role of one or more limiting substrates, we will consider here situations where these environmental variables will be, if not optimal, at least constant with values somehow "ideal" for microorganisms;

– as it is very often the case, if a substrate is limiting at low concentration, it can also prove toxic when its concentration becomes significant in the medium. In 1968 [AND 68], Andrews has suggested an expression for $\mu(s)$ involving three parameters to describe the growth rate of a microorganism limited by a low concentration of substrate but inhibited when the concentration becomes significant [HAL 30]. This function is written $\mu(s) = \mu_0 \frac{s}{s+K_S+\frac{s^2}{K_I}}$ where μ_0 and K_S are, respectively, the maximum growth rate and the semi-saturation constant in the absence of inhibition and K_I the inhibition constant. We will see that the choice of this kinetics leads to very significant changes in the qualitative properties of biological models. It is particularly interesting to note here that this function actually describes an indirect inhibition phenomenon. Similarly to the Monod equation, this equation is not a law of nature but expresses the fact that at high concentrations, the complex mechanisms involved in fact cause a change of pH and that it is this variation of pH which has, *in fine*, a consequence on growth. The Haldane function[2] thus perfectly illustrates the manner in which very complex phenomena can be reduced to simple modeling by adopting a "macroscopic" point of view – at the population level. The fact remains that if we adopt this very integrating vision, the range of validity of the developed model is limited and that it may prove necessary to write more complicated models capable, for example, of explicitly describing the relationship between biological growth and pH variation in the reactors. This reason has driven us to add at the end of this chapter a section called "Towards a little more realism" in which we give elements for the modeling in order to approach models closer to reality;

– if the previous kinetics do only involve the substrate concentration – they will be known as "substrate-" or "resource-" dependent; some authors have pointed out that the experimental data obtained from complex microbial ecosystems (typically in

2 It should be noted that this is the designation which is adopted in bioengineering, although it was Andrews who, inspired by the function proposed by Haldane to describe enzymatic phenomena, suggested the form that we have presented for biological reactions inhibited by substrate.

"mixed cultures") were better reproduced by making use of kinetics depending not only on substrate density, but also on biomass density. This is the reason why, in this case, this will be referred to as of "density-dependent" kinetics. The so-called Contois function is thus written $\mu(s,x) = \mu_{max}\frac{s}{s+K_S x}$. Its particularity is that it decreases when the biomass concentration increases. This function is also called "ratio-dependent" because it is rewritten in the form $\mu(\frac{s}{x}) = \mu_{max}\frac{\frac{s}{x}}{\frac{s}{x}+K_S}$. Similarly to the previous case of inhibition, this function is very helpful when it comes to account for complex aggregation phenomena such as biomass structuring in flocs. In effect, in this case, the part of biomass that is located in the core of the flocs receives the substrate by diffusion only, hence a strong limitation of growth: it follows that at a given microbial population, the overall growth is lower in comparison to a situation in which biomass would not be structured into flocs (see [LOB 06a, HAE 07] or still [HAR 07]). It is interesting to note that almost all the growth functions have long been studied in distinct disciplinary fields (typically in general ecology and microbiology) until these fields converge today, especially in microbial ecology, into a discipline that emerged in the early 2000s (see [JOS 00]). Again, we will see that the properties of the models are modified in a very significant manner when these kinetics are utilized.

1.2.5. *The benefits of the chemostat*

We recall here that the chemostat is originally an experimental device used to study microbial growth and possesses the principal characteristics of continuously operating and ensuring a homogeneous growth medium. The microbial ecosystem – whether implementing a pure or mixed culture – is the functional core of any biological reactor. In the presence of fixed environmental conditions and provided a structure of adequate growth function and a fine identification of its parameters, the publications available in the literature show that the model of the chemostat reproduces the experimental reality particularly accurately by considering all or part of the present ecosystem as functional populations (see [GRA 72] or [GRA 75]). Therefore, it appeared legitimate to make use of it as a "building block", so to speak, in order to study more complex situations, particularly related to the dynamics of diversity. As a result, as early as the 1960s, shortly after its invention, the device has been modernized and has become a prominent tool in microbiology laboratories to characterize all kinds of microbes. However, the "model of the chemostat" as a mathematical object was seen as an "formal entity", exposed in the field of general ecology to theoretically study the properties of trophic chains, questions related to microbial interactions or even to the genesis and the maintenance of diversity. It enjoyed his moment of glory in the 1980s with Hansen and Hubbell's works dedicated to "competitive exclusion", see [HAN 80] – to which we will return in detail later on (see Chapter 3) – before the growth in molecular biology that overshadowed it for a while in biology laboratories. Technically well understood nowadays by engineers and laboratory technicians, it has gained a renewed interest

since the 2000s because it satisfies a number of formalization requirements expressed in the field of microbial ecology (see [HOS 05]), to such an extent that microbial ecosystems have today become models for general ecology (see [JES 04]). Moreover, it is still the subject of studies as a mathematical object to the point that we can now say that it constitutes a branch of applied mathematics, certainly modest, but very active. Furthermore, it proposes a formal framework called "theory of the chemostat" centered around a small well-identified community of mathematicians. Finally, the evidence presented above, borrowing the formalism of process engineering, shows that it can actually be considered to constitute a relevant "building block" to move beyond the pure simulation approach and make it possible to address the study of models of complex biological processes in a systematic and generic manner.

The next section presents the essential elements for the understanding of more comprehensive models used in process engineering, notably when it is desirable to model the pH and gas-liquid exchanges. In addition, it specifies a number of experimental constraints useful to know to somehow measure the distance that may exist between the model of the chemostat and the experimental device that it models. This section is not necessary for readers solely interested in the chemostat as a mathematical object. Therefore, they may now skip to Chapter 2.

1.3. Toward "a little more" realism

1.3.1. *Extensions*

The systematic approach that has just been presented (description of the reaction scheme and the continuous dynamic equations of biological reactions) can be expanded to describe more complex biological systems, namely to taking into consideration a scheme involving more reactions, species and/or processes. Consequently, in water treatment by biological means, it is not uncommon to see models requiring dozens of variables paired with one another *via* a scheme implicating biological reactions simultaneously taking place in cascade and/or in parallel. Because expressing this type of system as equations can prove to be very tedious, it is recommended in such situations to use the so-called Gujer matrices.

The Gujer matrix merely describes the reactive part of the biological dynamics and as such transport terms do not appear therein. It is referred to as a matrix, but is actually presented in the form of a table in which can be found:

– in the first column, the set of processes that have to be modeled;

– on the first row, the set of all states (or components) of the system;

– in the last column, the set of kinetics.

Each element of the table (or matrix) contains a yield coefficient. To illustrate this explanation, consider the biological system consisting of the scheme [1.3]–[1.4]. This scheme involves two processes (growth and mortality), three states (s, x and x_d) and two kinetics (one for each process). The Gujer matrix of this system comprises two rows and three columns and is represented in Table 1.1.

Process	x	s	x_d	Kinetics
Growth of x	1	$-\frac{1}{Y(\cdot)}$		$\mu(s)x$
Mortality of x	-1		1	$k_d x$

Table 1.1. *Example of a Gujer matrix for the biological system whose reaction scheme is given by [1.3]–[1.4]*

The representation by means of the Gujer matrix presents several interests. First, it provides a means to test the model "balancing" in the sense where the mass balance, if the variables are all expressed in the same unit, should be able to "be balanced" even if this means adding matter compartments. This closure is due to the fact that what disappears from a compartment must necessarily appear in another compartment. Each row represents a process, balancing is simply tested by verifying that the sum of the components of a row is indeed zero. In the example below, this is the case only for the mortality process but not for growth: it is effectively verified that $1 - \frac{1}{Y(\cdot)} \neq 0$ (unless $Y(\cdot)$ is equal to one which is excluded for the reasons that we already have exposed in previous discussions). In fact, this is explained by the fact that we have included in the model only the result of the part of s which was used for growth without considering its complement, typically used for the creation of the energy required for this growth. Without getting into complicated metabolic details, this model could thus be completed by adding a CO_2 compartment whose excretion by the cell is representative of this energy produced. The new matrix would then assume the form represented in Table 1.2.

Process	x	s	x_d	CO_2	Kinetics
Growth of x	1	$-\frac{1}{Y(\cdot)}$		$\frac{1-Y(\cdot)}{Y(\cdot)}$	$\mu(s)x$
Mortality of x	-1		1		$k_d x$

Table 1.2. *Example of a Gujer matrix for the biological system whose reaction scheme is given by [1.3]–[1.4] with closure of matter in the growth term*

Another advantage of this matrix notation is that it will be possible to deduce the dynamics of the variables from a mere reading of the Gujer matrix. In effect, let us

remember that the dynamics of a biological variable is the sum of two types of terms: one related to the modeling of the hydrodynamics (in the case of the chemostat, it designates a transportation term in $D(x_{in}^i - x_i)$) and a reaction term accounting for the formation and disappearance processes of the component under consideration *via* the various processes modeled. The Gujer matrix allows the immediate notation of the latter term in the following manner. For the component i, it is sufficient to obtain the sum of the elements contained in the i^{th} column of the matrix, multiplied, element by element, by the kinetics that can be found in the last column. Let us illustrate this procedure with the example in Table 1.2. The part of the dynamics of x related to growth is given by the sum of the yield coefficient 1 that multiplies the kinetics $\mu(s)x$ (the term associated with growth) added to the mortality term expressed by multiplying the yield coefficient -1 multiplied by the term $k_d x$. Therefrom, it is concluded that $\frac{dx}{dt} = \mu(s)x - k_d x$. In order to obtain the complete equation, the last thing to do is to add the transportation term to yield the dynamic $\frac{dx}{dt} = \mu(s)x - k_d x + D(x_{in} - x)$ or even $\frac{dx}{dt} = \mu(s)x - k_d x - Dx$ if $x_{in} = X_{in} = 0$.

It is important to note that this type of extension – the introduction of new processes in ecosystem models – can be complemented by a more comprehensive description of biological reactions, which takes the form of a scheme where multiple reactions take place simultaneously in parallel and/or cascading, analogously to what is obtained in the case of a trophic chain. Thus, for example, anaerobic digestion, which is the transformation of organic matter into biogas (a mixture of methane and carbon dioxide), is achieved by a microbial consortium involving a very large number of groups of microorganisms within which groups of molecules are transformed into a certain number of products that themselves appear to be the substrate(s) of other groups of microbes. To cite only one example, the AMOCO model[3] or AM2[4], see [BER 01], thus involves two families of molecules combined into two variables, respectively, denoted by s_1 and s_2. It further presupposes a reaction scheme in two stages where a consortium of microorganisms x_1 transforms s_1 into s_2 which in turn is transformed by a consortium x_2 into biogas.

Now that we have presented the main tools to formalize a biological reaction from a perspective that could be qualified as being "macroscopic", the following section is dedicated to the modeling of physicochemical equilibria. Although in the remainder of this book, we do not specifically study this type of model, necessarily more complex than the "simple model of the chemostat", with this complement, we will be able to approximate the description of reality. This will be achieved by establishing a link between the description of the biological phenomena and physicochemical equilibria that govern, in particular, the pH, which is a key variable

3 From the name of the European project in which it has been developed.

4 For *Acidogenesis Methanogenesis Model*.

in the functioning of microbial ecosystems. It should be noted that at a fixed pH, we naturally fall back on the chemostat model...

1.3.2. *pH and physicochemical equilibria*

Chemistry tells us that most of the molecules that we immerse in water dissociate in several "species" (here taken within the meaning of chemistry) and that the forms they assume are in equilibrium in the medium depending on the pH. In addition, when it is said that a microorganism needs a substrate, for example nitrogen, this does not specify that it is usually able to assimilate it in ammonium form only and that, in another form, the substrate can be toxic. So far, we have performed balances on the total quantity of elements present in the medium while disregarding the form under which these elements were found. Nonetheless, this form depends on the pH. Thus, for example, nitrogen that is added to water is present in NH_4^+ form (acid, of concentration $[NH_4^+]$) and NH_3 (base, of concentration $[NH_3]$) which form what is called an acid-base pair. In other words, in water, the two following reactions simultaneously occur:

$$\begin{cases} NH_4^+ + H_2O \Rightarrow NH_3 + H_3O^+ \\ NH_3 + H_2O \Rightarrow NH_4^+ + OH^- \end{cases} \quad [1.11]$$

in which the ratio $\frac{[NH_3]}{[NH_4^+]}$ is related to the pH by the equilibrium formula $pH = pK_a + log(\frac{[NH_3]}{[NH_4^+]})$ where pK_a is a constant (experimentally determined within given environmental conditions; equal to 9.2 in normal conditions of temperature and pressure, which means that when the ratio of the two concentrations is equal, the pH is equal to 9.2).

At a pH greater than 9.2, the nitrogen will appear more in NH_3 form while at a lower pH, nitrogen will be more in NH_4^+ form. However, the modeling of these phenomena can be complicated because of several factors:

– some molecules are in equilibrium in several liquid, aqueous and possibly solid forms (*via* precipitation processes), these equilibria being not only dependent on the pH but also of other environmental variables such as temperature and pressure;

– at lower pH (respectively, larger), some chemical species can again be dissociated and it is then necessary to consider new variables corresponding to these new species if the aim is to correctly model the phenomena being addressed. This new species is itself in equilibrium with the two other forms that were considered until then: it results, therefore, that we are not considering an equilibrium equation but two reactions paired to one another by means of the pH.

Let us illustrate these two situations. First, we recall the case of nitrogen. Previously, we have deliberately omitted to specify that NH_3 is actually a gas whose dissolved part is in equilibrium with its gaseous form. Their respective proportions obey Henry's law which states that $[NH_{3_{aq}}] = K_{NH_3} * P_{NH_3}$, where K_{NH_3} is Henry's constant whose value depends on the gas, on the liquid and on environmental conditions, while P_{NH_3} is the partial pressure of the gas. It is paramount to realize here that the constants that appear in the various equations to which we reference are experimental values obtained for pure bodies. Since they cannot test the infinity of situations that may arise in practice, users will often merely limit themselves to these data and consider that the behavior in mixture is the result of the sum of the behavior of each of the elements if they were considered on their own in the medium. To illustrate the second situation, we will consider the chemistry of inorganic carbon (for details, see [COP 96] from which we extract this example). When dissolved, carbon dioxide is a "diacid", precisely because it disassociates twice, first into hydrogenocarbonate ions HCO_3^- (analogously to what has been written for nitrogen, this first equilibrium is governed by the equilibrium equation [1.12]), and a second time into carbonate ions CO_3^{2-} governed by the equilibrium equation [1.13]. If we denote the total carbon concentration (denoted by TC), we have $TC = [CO_{2,dissolved}] + [HCO_3^-] + [CO_3^{2-}]$ and the concentrations of the different forms of inorganic carbon are related to each other by equations [1.14]:

$$\frac{[HCO_3^-][H^+]}{[CO_{2,dissolved}]} = K_1 \qquad [1.12]$$

$$\frac{[CO_3^{2-}][H^+]}{[CO_{2,dissolved}]} = K_2 \qquad [1.13]$$

$$\begin{cases} [CO_{2,dissolved}] &= \frac{[H^+]^2}{[H^+]^2+K_1[H^+]+K_1K_2}TC \\ [HCO_3^-] &= \frac{K_1[H^+]}{[H^+]^2+K_1[H^+]+K_1K_2}TC \\ [CO_3^{2-}] &= \frac{K_1K_2}{[H^+]^2+K_1[H^+]+K_1K_2}TC \end{cases} \qquad [1.14]$$

the ratios $\frac{[CO_{2,dissolved}]}{CT}$, $\frac{[HCO_3^-]}{CT}$ and $\frac{[CO_3^{2-}]}{CT}$ depending only on the pH.

Having achieved a balance with TC, we have a system of four equations with four unknowns. Supposing that dissociation constants K_1 and K_2 are known, it is therefore possible to calculate the concentrations of the different forms of inorganic carbon and therefrom deduce the medium pH. In practice, in order to model the pH within a biological reaction, assuming that dissociations are very fast compared to

biological reactions, algebro-differential models are thus considered in which all the physicochemical equilibriums of existing species are expressed to thereof deduce the concentration of H^+ ions and therefore the pH. It should be noted that these are the considerations that will usually drive engineers to limit the range of validity of the models developed.

1.3.3. *Spatialization*

Previously, we have only mentioned situations in which the enclosure of the reactor was homogeneous. However, it is easily understandable that the more significant the volume of a reactor is going to be, the more this homogeneity condition is likely to be questioned. In addition, if we broaden the "formalization field of the chemostat to the description of natural ecosystems", then there are many situations in which the structuring of the natural space can be seen as more or less large – homogeneous – volumes connecting each others by flows of matter and/or energy. If we have a perfect knowledge of the dynamic behavior of each individual entity, the interest in formalizing this natural space as a network of interconnected chemostats is immediately understood. Note that if we consider flows of matter – whose intensity can be varied – going from a reactor A to a reactor B and *vice-versa*, we are confronted with a situation in which diffusion phenomena can be studied. The first configurations involving interconnected chemostats are known under the name of "gradostat" and have been proposed as early as the 1970s to simulate an environment where gradients of concentration of a limiting substrate can be observed as is often the case in a natural space (see [COO 73] or [LOV 79]). By adopting this approach, it is possible to represent numerous non-homogeneous situations by a network of interconnected reactors. The originality of these approaches is to avoid having to write partial derivative equations, which are more difficult to manipulate than a differential system even if the latter is of large dimension. The properties of the "chemostat" as a basic entity can then be "propagated" in the network, for example by simulations, and the properties emerging of the whole studied at least numerically. It is also interesting to point out that if the biological part is forgotten, these approaches that consist of considering networks of reactors (these are then essentially cascades of reactors in which the output of one is the input of the other, which are studied) have been used in the 1950s to study flows in chemical reactors. In particular, these networks have been addressed precisely to "approximate" the hydrodynamic behavior of non-homogeneous reactors, also called plugflow reactors, which is one of the "ideal" reactors of process engineering (see [CHO 59, CHO 60] or further [CHO 61]). In addition, such configurations have been proposed to bring forward ratio-dependent growths (see [ARD 92]) or still to model biofilm reactors (see [ESC 05]). In all these situations, flows – the flow rates between the different chemostats of the networks under consideration – are *a priori* constant. Already very rich in terms of dynamics, considering the chemostat or a network of chemostats in a

context where flows that connect them vary opens very interesting new perspectives as we will see in the next section.

1.3.4. *Recent developments*

At the present moment, the world of microbial ecology is going through a real revolution due to advances in molecular biology. That is how, from the research for the comprehension of evolution and cellular regulation mechanisms, a new disciplinary field called "systems biology" has recently emerged. This branch of science, basically multidisciplinary, seeks to understand the cellular mechanisms at the basis of the functioning of living cells. Its objective is clearly visible: to be able in the coming years to propose an "in silico" cell mimicking in every aspect the functioning of a living cell. This would be achieved by simulating its growth from reading its DNA until its division into two daughter cells. One of the difficulties that confronts researchers is to be able to study these cells within stationary environments, which a chemostat precisely allows. Associated with high-frequency sampling of all the characteristics of the medium but also of the internal cellular metabolites and with the use of all the "omics" measures available, new experimental devices have emerged, based on the principle of the chemostat (homogeneous and operating continuously). They are supposed to be able to provide researchers with the set of data that includes the necessary information for the understanding of studied phenomena. They take advantage not only of the fact that chemostats actually enable equilibria to be obtained, but also that these equilibria can be interacted upon *via* actuators. Operating then in a dynamic mode, they are called "changestats", "accelerostats" or still "decelerostats". One can, for example, distinguish the "turbidostat" (operation with controlled cells concentration), the "A-stat" or the "in-stat" (operations in which a succession of pseudo-equilibriums are obtained before the inflow rate is increased or on the contrary decreased, either continuously or abruptly by means of applying a level), "auxo-stat" (operation with controlled oxygen concentration), etc. In this new context, the conventional chemostat becomes a particular case called "D-stat" (see [ADA 15]).

It seems particularly relevant to make two observations here. The first is that although these devices manifestly appear as carrying a strong application potential, their theoretical study remains to be achieved and may hold a few surprises. For example, Lobry *et al.* have considered a simple situation in which two species are in competition on a limiting resource within a chemostat subjected to a slow variation in the inflow rate. They have established that the model describing this device presented a phenomenon called "stability loss delay". This is a property of dynamical systems that allows the species that finally loses the competition able to sustain themselves "longer than expected in the system" even though the feeding flow rate reaches a value that is unfavorable (see [LOB 09] or [LOB 13]). The second observation is that the devices to which we are referring here are not as recent as it might seem, since a

number of them were proposed as early as the 1990s. However, it is rather their utilization in the context of systems biology and, as a result, the renewed interest in the chemostat that is interesting to point out here (see [HOS 05] or still [ZIV 13]).