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A Structured Model for the Simulation of Bioreactors Under Transient Conditions

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Modeling the transient behavior of continuous culture is of primary importance for the scale-up of biological processes. Spatial heterogeneities increase with the reactor size and micro-organisms have to cope with a fluctuating environment along their trajectories within the bioreactor. In this article, a structured model for bioreactions expressed in terms of biological extensive variables is proposed. A biological variable is introduced to calculate the growth rate of the population. The value is updated on the basis of the difference between the composition in the liquid and biotic phase. The structured model is able to predict the transient behavior of different continuous cultures subject to various drastic perturbations. This performance is obtained with a minimum increase in the standard unstructured model complexity (one additional time constant). In the final part, the consequences of decoupling the growth rate from the substrate uptake rate are discussed. © 2009 American Institute of Chemical Engineers *AIChE J,* 55: 2973–2984, 2009
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Introduction

Biological systems are known to be highly sensitive to any fluctuations in their environment. Building models dedicated to the simulation and the prediction of the dynamic response of biological systems to unsteady state conditions is therefore a major objective in the domain of bioprocess engineering. Such models must be constituted essentially by:

(i) A hydrodynamic model of the reactor. This model can be an ideal continuous or plug flow reactor, a compartment model[1–3] or a Computational Fluid Dynamics based description of the flow field.[4]

(ii) A dynamic model for the bioreactions that defines both the local intensities of the bioreactions with respect to the physiological state and the dynamic adaptation of the cells to the fluctuations in their local environmental conditions.

The application area concerns the response of lab-scale bioreactors to sudden shifts in the inlet concentration or in the dilution rate as well as the simulation of the performance of large scale, heterogeneous, bioreactors. On a laboratory scale, the duration, magnitude, and frequency of the fluctuations can be controlled.[5] On large scale processes, these fluctuations are due to the spatial heterogeneity of the hydrodynamic and concentration fields.[4,6] The characteristics of the fluctuating signal encountered by the cells along their trajectory within the reactor are essentially unpredictable but can be approached by numerical simulation.[7–9] However, as pointed out by Enfors et al.,[10] the global performance of a large scale bioreactor is an integrated consequence of all these fluctuations on all micro-organisms. Thus, the modeling of the dynamic response of biological systems is equally crucial for a successful scale-up of bioprocesses.

It is now generally admitted that the so called unstructured models are unsuited to the situations listed above. Reasons for this failure are numerous and have already been cited many times.[4,11–17] Basically, unstructured models are issued from conservation (mass balance) equations over the entire reactor. They result from a double averaging (in time and space) of a wide variety of intracellular reactions and lead to a description of the mean behavior of the population in terms of an apparent kinetic rate. Thus, unstructured models make no distinction between individuals, and they are suited

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to situations where a pseudo steady state can be defined at the reactor scale. This is the case for batch experiments where the changes in the liquid phase are only due to the bioreaction itself, for continuous cultures at steady state and for fed-batch cultures in which the addition of substrate is tuned to meet the biological needs.[13] The formalism of unstructured models implies an immediate adaptation of the growth rate in response to a change in the substrate concentration because of the algebraic relation between μ and S. The predictive capacity of unstructured models under transient conditions is hampered by the fact that the steady state data of substrate uptake at different dilution rates depend on the induction of different transporter systems.[4] Indeed many experiments show that partial decoupling between assimilation and growth occurs in response to a sudden change in the environmental conditions.[5,18–20] Therefore, when spatial heterogeneity exists within the bioreactor or when a homogeneous bioreactor is subjected to sudden shifts in concentration or dilution rate, unstructured models have proved to be inefficient for the process control of a steady state culture in a chemostat,[14] the prediction of the transient response[15,21,22] or the transient approach to the steady state.[23]

On the other hand, structured models consider the concentration of intracellular material to describe the cell functioning as well as the values of the mass fluxes exchanged with the environment. A presentation of structured models is proposed by Nielsen and Villadsen[13] in a review article. Complements including metabolic, cybernetic, and mass balance population models can be found in the reviews of Llaneras and Pico,[24] Ramkrishna and Mahoney,[25] Varner and Ramkrishna,[26] and Fredricskon.[27] In a structured model some insight into the intracellular composition is included, and the description of the cell factory is now given in terms of internal variables. Depending on the complexity of the biological systems and on our level of knowledge, the choice of the internal variables can be more or less biologically sound.[28] This modeling approach has produced a large number of submodels the classification of which is not easy to establish. Historically, one can distinguish multiple types of models such as:

(i) Adaptation or time delay structured models[14,19,21]
(ii) Compartment models[28,29]
(iii) Cybernetic model[23,28]
(iv) Metabolic models.[30–32]

Whereas compartment and cybernetic models are well suited for the simulation of biological systems under transient conditions, metabolic models in general are based on two characteristics: the use of a metabolic network and the assumption of a pseudosteady state[24,33] which limits their applicability in the domain of transient simulations.[26] Recently, however, efforts were made to produce dynamic metabolic models for a given pathway.[34,35] The number of intracellular components considered in these models is important (up to 27) so is the number of enzymatic reactions (>30). This requires the determination of a kinetic law for each enzymatic reaction and the identification of the relevant kinetic parameters.[36] Finally, the metabolic modeling is generally focused on a few processes that are of interest in the current investigation, and it is highly dependent on the assumptions used in the construction of the network and the simplifications made. Thus, the predictive power of metabolic models is limited.[26] The latest development in the modeling of the transient response of microorganisms refer to a combination of cybernetic and metabolic models.[37,38]

In this brief overview of unstructured and structured models, the cell population is treated as an average single cell i.e., no distinction is made between individuals. When some heterogeneity exists in the reactor, it is likely that it produces heterogeneity among the population of cells.[39] The mass balance population concept therefore appears as the most natural and promising tool to address the issue of dynamic modeling of cell population behavior. A review of the applications of the population balance technique in the domain of cell population modeling is beyond the scope of this article and details can be found in the works of Fredrickson,[27] Ramkrishna and Mahoney,[25] Mantzaris and Daoutidis,[40] and Henson.[41–43] However, two points may be recalled:

(i) The number of internal variables that can be considered is rather small compared with the total number of cell components and only a few of them are actually measured.[44]
(ii) There is a lack of model for the description of the rate of change of internal state variable with respect to both other internal variables and environmental variables.[45]

From this literature survey, one can draw up the conclusion that there is no simple generic model dedicated to the simulation of multispecies multistrain biological systems under transient conditions that can be coupled with a hydrodynamic model. Modifications in the latest version of the Activated Sludge Model no. 3 still make use of a time delay function to model the transient behavior in response to environmental changes.[19] The main drawback of the time delay structured models is that one has to know the moment when a perturbation occurs to trigger the adaptation process. This seriously affects the predictive capacities of such models.

Another conclusion is that there are many ways in which a micro-organism responds to a change in its environment, and they cannot be addressed by a single model. So, one will restrict the purpose of the modeling study to the effects on the growth rate in continuous cultures subject to a sudden change in the concentration or dilution rate. In such a situation, the experimental observations show that the response is related to:

- The magnitude of the perturbation.[14,46]
- The history of the culture or the initial conditions before the perturbation.[17,22,46]
- The sign of the perturbation: O'Neil and Lyberatos[47] have identified different time constants for step-up and step-down experiments performed on continuous cultures of *Saccharomyces cerevisiae*.

Unless moderate perturbations are applied, there is no immediate adaptation of the culture. Moreover, Leegwater et al.[20] pointed out that there is a partial decoupling between catabolism and anabolism in response to the relief of a carbon substrate limitation. The extra amount of substrate is assimilated instantaneously but the conversion into new cells is not immediate.[5,19]

This article focuses on a model suitable for homogeneous bioreactors subject to perturbations. The biological model formulation enables a straightforward coupling with transport equations for the simulation of multistage or heterogeneous bioreactors. The model presented here is mostly dedicated to

the situations where a reference to a detailed knowledge of the metabolism is not possible either because it is not known for the strain studied or there are multiple strains in interaction (e.g., in biological waste water treatment processes). However, the proposed model is first used to simulate pure strain batch and continuous cultures subject to concentration and dilution rate shifts to identify the time constants introduced.

Model Framework

The model operates at the scale of the reactor which is assumed to be homogeneous, so the hydrodynamic part of the model reduces to mass balance equations written over the entire reactor of constant volume V:

$$\frac{dX}{dt} = \frac{Q}{V}(X_{in} - X) + r_X \text{ with } r_X = \mu \cdot X \tag{1}$$

$$\frac{dS}{dt} = \frac{Q}{V}(S_{in} - S) + r_S \text{ with } r_S = -\frac{1}{Ysx}\mu \cdot X \tag{2}$$

$$\frac{dC_{O_2}}{dt} = \frac{Q}{V}(C_{o_{2in}} - C_{o_2}) + r_{O2} + K_L \cdot a\left(C_{o_2^*} - C_{o_2}\right) \quad \text{with} \quad r_{O2} = -\frac{1}{Yox}\mu \cdot X \tag{3}$$

The biological model presented in this article is a time delay structured model: it uses internal variables, e_i, to describe the physiological state of the biological phase and adaptation functions for these variables. These internal variables are related to the composition of the cell. The internal variables are used to compute the growth rate that the population can achieve owing to its own capacities. It is referred to as the biological growth rate μ^b and a standard Monod formulation is used to define this biological growth rate as:

$$\mu^b = \mu^0_{\max} \prod_i \frac{e_i}{k_{e_i} + e_i} \tag{4}$$

In most unstructured models for biological reaction the growth rate is expressed directly as a function of the concentrations in the liquid phase.

$$\mu^0 = \mu^0_{\max} \prod_j \frac{S_j}{K_{S_j} + S_j} \tag{5}$$

In this article, μ^0 is regarded as an equilibrium growth rate. It corresponds to the growth rate when the population of cells is in equilibrium with the environment. One of the basic ideas of this model is that these two growth rates can be different but the biological system always tends toward the equilibrium defined as the equality of the capacities of the cell and the potential offered by the environment. A key point is that this formulation decouples the growth rate from the local values of the concentrations in the environment. This equilibrium is reached asymptotically in a chemostat for long cultivation time and one gets $\mu^0 = \mu^b$. Under transient conditions, however, the instantaneous growth rate of the culture, μ^a, is computed as the minimum of the biological and equilibrium growth rate and is used in Eqs. 1–3.

$$\mu^a = \min(\mu^b, \mu^0) \tag{6}$$

The biological growth rate may be smaller than the equilibrium growth rate because the population has not yet performed the necessary adaptations e.g., the synthesis of a higher quantity of a key enzyme. This situation typically occurs in a step-up experiment when a sudden increase of the substrate concentration in the feed leads to an increase of the substrate concentration in the liquid phase of the reactor. On the opposite, it is assumed that when the concentration falls rapidly as in the step-down experiment, there is a reduced transfer of substrate from the liquid phase to the biotic phase, which limits the growth rate of the biomass. Such a situation corresponds to a substrate limited culture, so μ^0 appears as a good estimate of the growth rate.

A single variable formulation is used for the biomass so one assumes that the quantity of e is the same in all cells. An equilibrium constant K_{eq}, defined as the ratio between e and S at equilibrium could be introduced but indeed, the value of the internal saturation constant k_e can be set to K_S without any loss in generality ($K_{\text{eq}} = 1$). Although its chemical identity is not specified, the biological variable e is taken as an extensive variable, expressed in arbitrary units per gram of biomass, so a conservation equation for e is written as:

$$\frac{d(X \cdot e \cdot V)}{dt} = Q \cdot X \cdot e|_{in} - Q \cdot X \cdot e + r_e \cdot V + A_e \cdot X \cdot V \tag{7}$$

If one assumes that the physiological state variable, e, is conserved through the growth process, then one can write r_e as:

$$r_e = \mu^a \cdot X \cdot e \tag{8}$$

The originality of this modeling lies in the last term on the right hand side, A_e, which represents the specific rate of production of e as the consequence of the biological response to the concentration fluctuations in the environment, say adaptation. In this approach, it is proposed that the detection by the population of some changes in the environment may result from a discrepancy between the external and the internal mass fluxes at the liquid–biotic interface. More exactly, one may consider the rate of transfer from the liquid phase and the rate of utilization of the substrate for growth. At a steady state, these two fluxes are equal but in dynamic situations, there is no reason why theses two fluxes should be equal all through. The adaptation rate of a population of cells is driven by the occurrence of a discrepancy between the fluxes in the liquid phase and the biological phase.

$$A_e = \delta q_+(e^* - e)\frac{2e}{e^* + e} + (1 - \delta)q_-(e^* - e) \qquad \delta = 1 \text{ if } e^* > e \text{ and } \delta = 0 \text{ otherwise} \tag{9}$$

The first term on the right hand side of Eq. 9 is active when the environmental conditions are favorable to an increase of the specific growth rate (i.e., $\delta = 1$). The first factor, $q_+(e^* - e)$, has the dimension of a flux and is the product of a rate constant by a driving force. The driving

force of adaptation is then taken as the difference between the instantaneous value of e and the value e^* that would be reached in the same environment at equilibrium. So, the biological phase variables are dynamically adapted with respect to the concentration difference between the liquid and biological phases. The same formalism is used for the modeling of two-phase mass transfer phenomena. The second factor, a hyperbolic function of e, has the form of an enzymatic reaction term. It depicts the fact that additional quantities of e must be synthesized to metabolize the additional amount of substrate available. This function also accounts for a possible slowing down of the adaptation processes when $e^* \gg e$: the substrate is in large excess compared with the cell's needs. In such cases, although the substrate is assimilated, it is not necessarily used up for growth purposes and might even be excreted by the cell under another form (over-flow metabolism). The coefficient 2 ensures that this term goes to one when e goes to e^* that is to say, when a new equilibrium is reached.

The second term on the right hand side of Eq. 9 is active when environmental conditions are limiting i.e., when the value of the concentration in the liquid phase is lower than the corresponding internal capacity. Because the biological reaction is limited by the transfer from the liquid phase, the cell's capacity is not fully exploited and the model assumes that the regulation processes within the cell will tend to reduce this overcapacity. The second term on the right hand side models the depletion of the unexploited overcapacity under substrate limiting conditions. The second term is therefore essentially negative. A general formulation is chosen for the rate q which is made dependent on both growth and nongrowth associated processes.

$$q = \frac{1}{\tau} + \mu^b \tag{10}$$

τ is a time constant for nongrowth associated adaptation processes. The rate of growth associated processes is taken equal to the biological growth rate (Eq. 4). The biological growth rate is used in Eq. 10 because it is a more sensible estimate of the rate of intracellular reactions than μ^0, which can also reflect external limitation by the transfer from the liquid phase as demonstrated by Merchuk and Asenjo.[48] In practice, two rates q_+ and q_- are defined, with a corresponding time constant τ_+ and τ_-. The subscript + means that the rate q_+ is used when $e < e^*$, i.e., adaptation will result in an increase of e. The subscript — means that the rate q_- is used when $e > e^*$, i.e., adaptation will result in a decrease of e.

Typically, A_e goes to zero at steady state, and e reaches an equilibrium value $e = e^* = K_{eq}S$. Therefore, the growth rate calculated in Eq. 4 equals that of the unstructured model under the same conditions (same value of S). Thus, at steady state, the structured model formed by the set of Eqs. 1–10 leads to the same results as the standard unstructured model, defined by Eqs. 1–3 and 5. In the following, a comparison with existing data is used to identify the values of the time constants introduced in the structured model.

Comparison to Some Existing Models

The main features of the model are listed and compared with other approaches found in the literature.

- In the proposed form, the dynamics of adaptation depends on the initial conditions: values of e and μ^b. This feature is common to all structured models.[22] It also depends on the magnitude of the perturbation: the role of the hyperbolic function in Eq. 9 is to introduce inertia effects in the response.
- It will be seen in the model that, the dynamics is also conditioned by the biological characteristics of the organism (namely μ_{max}). This is consistent with the observations of Kätterer et al.[46]
- The discrepancy between the potential offered by the environment and the capacities of the cell is the driving force of the adaptation. At steady state, these two are at equilibrium ($\mu^0 = \mu^b \Rightarrow e^* = e$). It is not necessary to know when precisely a perturbation occurs because adaptation is automatically triggered by a shift from the steady state conditions. This is a limitation in the model of Young et al.,[11] Patarinska et al.,[14] and Sin et al.[19]
- The dynamics of adaptation is obtained without introducing arbitrary time delay functions $\mu(t)$ as done by Young et al.[11] and Chiam and Harris.[21] The adaptation function is not build on a continuous time differential equation for the intensive variable μ.[14,22,49] Note that with such models the coupling with transport equations to model the hydrodynamics of heterogeneous bioreactors is not possible since one can not write a conservation equation for an intensive variable.

Comparison to Experimental Data

The batch simulation

In this part, the structured model formed by the set of Eqs. 1–10, is compared with the standard unstructured model which is known to correctly represent the batch experiment. The equations for the unstructured model are Eqs. 1–3 with μ given by Eq. 5. At first, it is assumed that the gas liquid transfer rate in the bioreactor is high so that oxygen is never a limiting factor. In this first step, a single limitation on the carbon substrate source is considered.

The strain chosen is *Escherichia coli*. The saturation constant ($K_S = 0.05$ g l^{-1}), the conversion yield ($Y_{SX} = 0.45$) and the maximum growth rate ($\mu_{max} = 0.6$ h^{-1}) are taken from Xu et al.[32] The value of q_+ is issued from the experimental observations of Zafiri et al.[49] which show that adaptation and growth proceed on a similar time scale. Rearranging their results, one estimates that

$$q_+ = 0.8\mu_{max} + \mu^b \text{ i.e., } \tau_+ = 1/0.8\mu_{max} \tag{11}$$

The initial guess for q_- is less easy to make, and it is first decided give q_- a value that is one order of magnitude higher than the value of q_+. This would imply that the biological variable e decreases at the same rate as the concentration in the liquid phase. Figure 1a, b compare the results of the simulation of a batch culture performed with the two models but with different initial values of e.

In Figure 1a, the initial value of e is set to e^*, the value at equilibrium with the liquid phase to mimic a population initially adapted to a glucose rich environment. It is seen in Figure 1a that the structured model produces the same

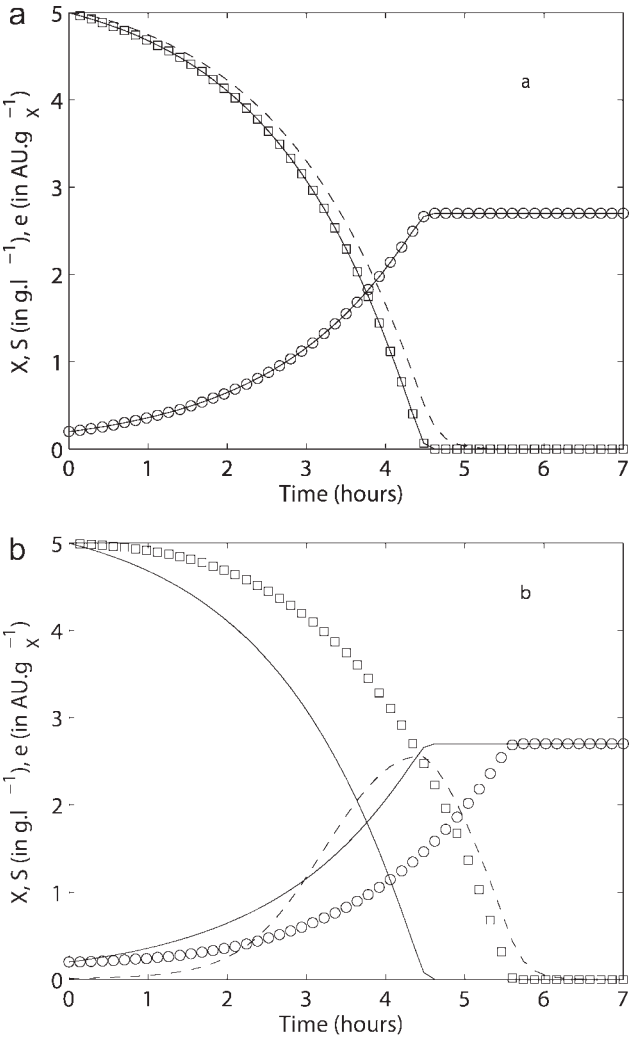

Figure 1. Batch culture of *E. coli* with an initially adapted biomass (a) and with an initially non-adapted biomass (b).

Continuous lines: unstructured model; symbols: structured model (□: glucose; ○: biomass); dashed line: internal variable

results as the unstructured model. The unstructured model is known to be suitable for the batch experiment because changes in the environment are due to the biological reaction solely. So the quasi-steady state hypothesis underlying the use of the unstructured model is always valid in a batch culture. The perfect match between the two models confirms the correct implementation of all equations in the Matlab® code that was developed.

In Figure 1b, the simulation is performed under the same conditions except for that the initial value of the state variable e is set to give $\mu^b\ (t=0) = \mu^0/5$. At the beginning of the culture, the population is unable to achieve the equilibrium growth rate, since the actual growth rate is controlled by the internal capacity of the cells which is low. The internal capacity progressively increases with time according to Eq. 9, which in turn leads to an increase of the biological growth rate. As soon as e becomes significantly higher than k_e ($t > 3$ h), the difference between the two growth rates, μ^b and μ^0, becomes negligible. From that point onward the culture dynamics are controlled by the concentration in the medium, and they are no longer limited by the physiological capacities of the cells. In comparison to the previous case studied (biomass initially adapted), one observes that the exponential growth phase at $\mu = \mu_{\max}$ is delayed (starts at $t =$ 3 h) and is shortened (2 h 30 instead of 4 h 30) when the biomass is initially nonadapted.

A sensitivity analysis on the value of the time constant τ_- has been performed. Indeed τ_- acts on the magnitude of the memory effect as it controls the depletion rate of the cell's capacity to use a substrate for growth. Three situations were examined. The results are presented in Figure 2.

(a) $1/\tau_- \gg \mu^b$, the decrease of the cell's capacity is due to either physical or nongrowth related biological phenomena. This could correspond for instance to a physical transfer from the cell to the medium, or an important shift in the cell metabolism. The results show that the cell capacity diminishes as fast as the concentration in the environment, so there is no memory effect. This behavior is in contradiction with experimental observation, since an addition of substrate just after its exhaustion would result in another adaptation period.

(b) $1/\tau_- \ll \mu^b$, the decrease of the capacity proceeds at a rate defined by μ^b (Eq. 4). This rate diminishes with e resulting in the cell capacity being preserved on a long time scale. Thus, an addition of substrate after a prolonged period of famine would result in an absence of delay in the biological response. This situation is not supported by experimental evidence.

(c) $1/\tau_- \approx \mu^b$, the regulation of the internal capacity is under the control of both growth and nongrowth related

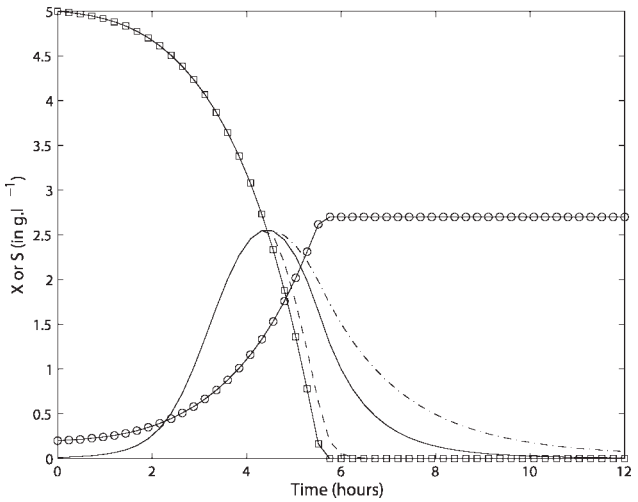

Figure 2. Sensitivity analysis on the parameter τ_- controlling the depletion rate of the internal capacity.

Continuous lines with symbols: concentrations in the liquid phase (□: glucose, ○: biomass); lines without symbols: internal capacity e. dashed line: fast depletion rate $1/\tau_- \gg \mu^b$; dotted line: slow depletion rate $1/\tau_- \ll \mu^b$; continuous line: $1/\tau_- \approx \mu_{\max}$. These latter three lines superimpose as long as $e < S$.

biological phenomena. The results presented in this case are obtained with $\tau_- = \tau_+ = 1/0.8\mu_{max}$. The internal capacity is maintained for about 2 h after the substrate exhaustion and goes to 0 within 4 h. Thus, an addition of substrate within 2 h following the substrate exhaustion would result in rapid consumption of the substrate associated with a high growth rate whereas the same addition 2 h later would result in a much slower consumption and a new adaptation period. Similar conclusions have been reported by Sonnleitner et al.[22]

It is therefore conceivable to conclude that the depletion of an internal capacity related to growth is likely to occur at the same rate as the growth rate, and, therefore, the value of τ_+ and τ_- are identical (continuous line). Of course these are only estimates that may serve as initial guess before an optimum value is found through a comparison to experimental data. The consequence is that the number of parameter of the structured model reduces to one: τ_+. In the following, all simulations are performed with a single time constant $\tau = 1/(0.8\ \mu_{max})$ which implies that Eq. 11 is used for both q_+ and q_-.

The chemostat simulations

The model has now been tested with three independent sets of experimental data. The first data set is for a single strain, yeast *Candida tropicalis*, cultivated in a chemostat and subjected to a sudden shift in the dilution rate.[46] The second one is taken from the work of Patarinska et al.[14] and O'Neil and Lyberatos[47] who have been working on continuous cultures of *Saccharomyces cerevisiae*. The third set of data is issued from the works of Gaudy and coworkers[50,51] and concerns a continuous culture of heterogeneous microbial population. The same data set has been used by Young et al.[11] to test their first order model.

Continuous Culture of Candida Tropicalis. Kätterer and coworkers have reported the data from continuous cultures of *Candida tropicalis* performed in a 3L reactor, equipped with a two stage stirrer rotating at 1000 rpm, under glucose limiting (concentration below 10 mg l^{-1}) and oxygen sufficient ($pO2 > 50\%$) conditions. After a batch growth phase, cells are transferred into a chemostat and cultivated at a dilution rate of 0.1 h^{-1} until a steady state is reached (e.g., during more than 7 volume changes). A step increase in the dilution rate from 0.1 to 0.42 h^{-1} is applied to the reactor. The experimental data and the simulations with the unstructured and the structured model are presented in Figure 3a, b. The biological characteristics of the strain are given in the cited paper ($\mu_{max} = 0.52\ h^{-1}$) and the saturation constant and yield were deduced from the steady state ($K_S = 10$ mg.l^{-1}, $Y_{SX} = 0.61$).

A good qualitative agreement is observed between the structured model and the experimental data. The main features of the dynamic response of the biological system are well represented. Interestingly, this qualitative agreement is obtained without any adjustment of the structured model parameter. The introduction of an inertia effect in the dynamic response of the biological system (Eq. 9) allows the prediction of washing-out after a sudden increase of the dilution rate. The reduction in the biomass concentration along with the increase of the substrate inflow causes a significant accumulation of substrate in the medium, which is also well predicted by the structured model. However, the unstructured model is unable to reflect neither the partial washing-out of the reactor, nor the associated accumulation of glucose during the transition between the two steady states.

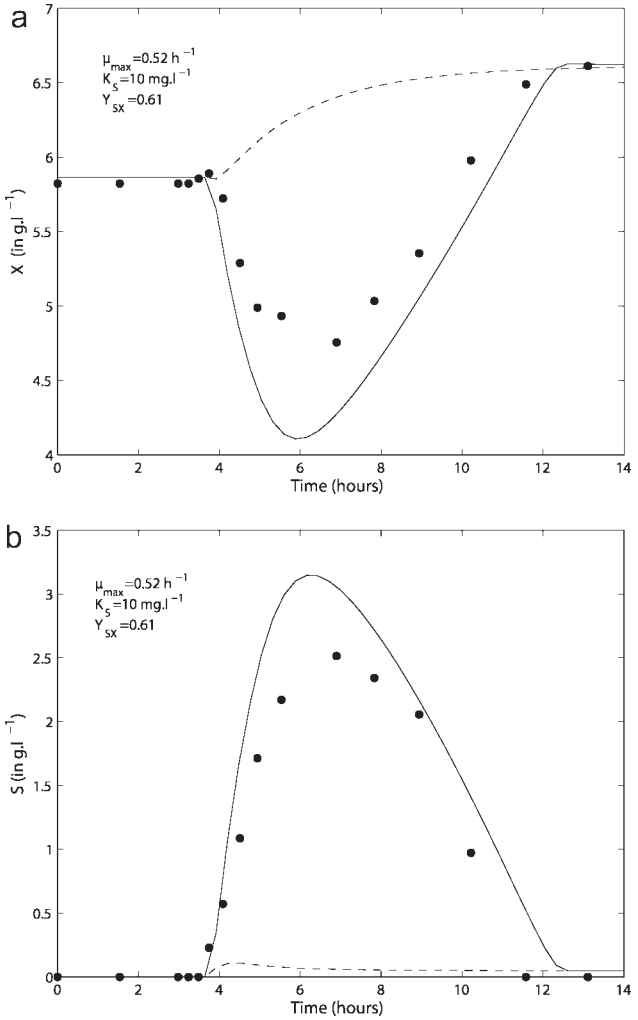

Figure 3. Transient response of a continuous culture of *Candida tropicalis* to a sudden shift in the dilution rate D = 0.1 to 0.4 h^{-1}; Biomass concentration (a), substrate concentration (b).

Dashed line: unstructured model; continuous line: structured model; ●: experimental data.

To predict the correct levels of biomass in the initial and final steady states, it has been necessary to introduce a relation $Y_{SX} = f(\mu^b)$, by using a Pirt's law where $Y_{SX,max} = 0.75$ and $m = 0.03$. However, it has been verified that the dynamics of the response is not affected by the use of a constant or variable yield coefficient. A variable yield coefficient essentially affects the shape (skewness) of the glucose accumulation curve. The observed change in the yield coefficient means that the specific glucose consumption rate and its partition between different metabolic pathways within the cell changes during the transient adaptation. A stationary law, based on steady state experiments, is used to account for the overall consequences of this dynamic behavior. Obviously, any kinetic

model based on global mass balance at the reactor scale is unable to describe precisely the metabolic rearrangement. During the transient response to a perturbation, the cells are not in equilibrium with their environment, and, therefore, it is not conceptually satisfactory to make use of a stationary law to set the instantaneous value of the yield coefficient. The fact that a stationary law is used to describe the dynamic rearrangement of the metabolism can explain the discrepancies between the model and the experimental data, especially for the substrate concentration curve.

Continuous Culture of Saccharomyces Cerevisiae. O'Neil and Lyberatos,[47] carried out experiments on continuous culture of *Saccharomyces cerevisiae*. They imposed step changes (positive and negative) for the concentration feed and dilution rate. They proposed a model based on a differential equation for the specific growth rate. Patarinska et al.[14] worked on the modeling of these experiments carried out upon a step change for the value of the dilution rate. These results were well represented by a time delay unstructured model, called S-type model. Full details can be found in the two articles mentioned previously but basically this S-type model defines the growth rate as a function of the biomass concentration and global variable w, defined as the weighted averaged of substrate concentrations. The variable w plays the same role as the variable e in our model; with the restriction that w is not defined by reference to the biological phase resulting in a model formulation that cannot be coupled to transport equation. The conservation equation for the biomass over the reactor is:

$$\frac{dX}{dt} = \mu(X, w)X - DX \tag{12}$$

The rate of change of the internal variable w is given by a first order differential equation:

$$\frac{dw}{dt} = \alpha(S - w) \tag{13}$$

So in this model, the adaptation function is a first order linear function with a time constant α. The authors have calculated the value of α through an error minimization procedure. The reported values of parameter α are in the range of [0.3–0.6]. This is consistent with the conclusions of O'Neil and Lyberatos who have pointed out that the value of α was different for step-up $\alpha \in [0.29–0.52]$ and step down experiments ($\alpha = 0.88$). Moreover, the values are different depending on the dilution rate: in step-up experiments of the feed concentration, a higher value for α is found when the dilution rate is high.

The comparison with our model is performed on the normalized adaptation rate: on the one hand A_e divided by e^* for the present model (Eq. 9)

$$\frac{A_e}{e^*} = \left[\delta q_+ \frac{2e}{e^* + e} + (1 - \delta)q_-\right] \frac{(e^* - e)}{e^*} \tag{14}$$

on the other hand, Eq. 13 divided by S for the S-type model:

$$\frac{1}{S}\frac{dw}{dt} = \alpha \frac{(S - w)}{S} \tag{15}$$

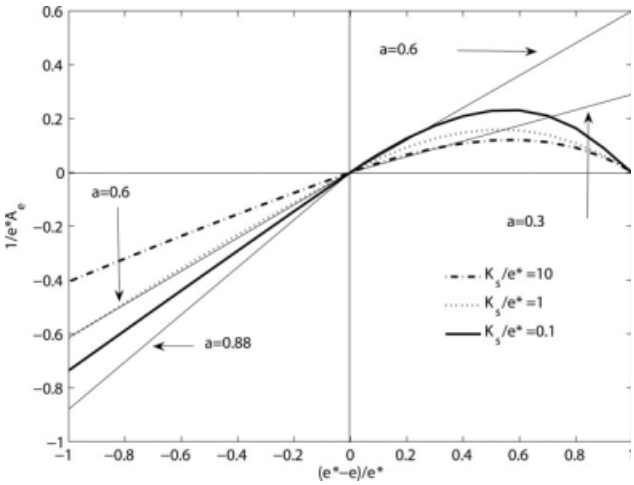

Figure 4. Normalized adaptation rates as a function of the normalized step magnitude.

Thin continuous lines: extreme values of the adaptation rates from Patarinska et al.[14] and O'Neil and Lyberatos.[47] Thick continuous, dotted and dashed lines: present model for different values of the parameter K_S/e^*: 0.1, 1, and 10, respectively.

The results are presented in Figure 4 where the value of the normalized adaptation rate is plotted against the normalized magnitude of the step $(e^* - e)/e^*$ or in an equivalent way $(S - w)/S$. In this figure, the results of the S-type model corresponding to the fitting of the different experimental data are represented by straight lines. The slope 0.3 is for the step-up experiment at low dilution rate. The slope 0.6 is for the step-up experiment at high dilution rate. The slope 0.88 is for the step-down experiment. The three curves are obtained with this model for different values of the parameter K_S/e^* ranging from 0.1 to 10. This ratio reflects the degree of limitation by the substrate before the perturbation. A small value means that the culture is not limited initially, whereas a high value means that a strong limitation exists.

For the step-up experiments, $e^* - e > 0$, the curves reflecting the adaptation rate calculated by the model reach the straight lines ($\alpha = 0.3$ and $\alpha = 0.6$) asymptotically when $e^* - e$ goes to 0. These curves are obtained respectively for $K_S/e^* = 0.1$, i.e., a culture which is initially characterized by a small growth rate (performed at a low dilution rate), and for $K_S/e^* = 10$, i.e., a culture close to its maximum growth rate (performed at a high dilution rate). Thus, for moderate steps (small positive values of $(e^* - e)/e^*$), the adaptation rate is highly dependent on the actual growth rate before the perturbation. The ability of the model to predict the differences in the apparent adaptation rate is due to the presence of μ^b in Eq. 11. Owing to this addition, there is no need to tune the time constant for each experiment; in our approach, τ is constant but q varies because of μ^b, which is dependent on the cell's capacity.

For the step down experiment, the $K_S/e^* = 0.1$ curve lies between the two straight lines $\alpha = 0.6$ and $\alpha = 0.88$. This is consistent with the fact that a step-down experiment is necessarily performed on a culture at a high dilution rate. So the ratio K_S/e^* is initially low, and the adaptation rate is high.

Thus, the proposed model is intrinsically in a position of reproducing without any further model structure adjustment the various dynamics observed experimentally for continuous cultures over a large range of dilution rates.

Continuous culture of activated sludge

In this section, the model has been tested against experimental data taken in the field of waste water treatment.[51] Contrary to the previous examples, activated sludge consists of a mixed culture of microorganisms. The experiment considered is a step increase in the concentration of the glucose feed from 450 to 1450 mg l^{-1} at constant dilution rate of 0.244 h^{-1}. The values of the kinetic parameters $\mu_{max} = 0.54$, $K_S = 0.1$ and $Y_{SX} = 0.64$ are those reported by Storer and Gaudy.[50] The results are presented in Figure 5a, b in terms of biomass and substrate concentration, respectively. A better fit has been achieved for biomass with a 10% increase of the maximum specific growth rate, i.e., $\mu_{max} = 0.6\ h^{-1}$. A constant yield is used although the growth rate actually varies because there are no experimental data that allow for the calculation of the parameters of a Pirt's law. In these figures, the values obtained with an unstructured model are represented by a dashed line. There again, the proposed model is capable of reproducing the data for biomass without any parameter adjustment, i.e., $\tau = 1/(0.8\ \mu_{max})$. This was also tested in the comparison with other data sets, which suggest that Eq. 11 may be a rather general one. In Figure 5b, the prediction of glucose concentration given by the models has been plotted against the measured COD and COD due to carbohydrate concentrations. The authors reported that carbohydrate is one of the end products elaborated in response to the shock. Whatever the model used, the magnitude and location of the peak in the glucose concentration are well predicted. The magnitude is slightly under estimated by the standard unstructured model and over estimated by the structured model. In contrast, the decreasing part of the curve after the peak is not well reproduced. One observes that the model prediction is rather good as long as the growth rate remains close to its initial value (up to $t = 4$ h). When the growth rate starts increasing significantly ($t > 4$ h), the glucose uptake rate also increases resulting in a faster decrease of the glucose concentration. This suggests that the yield coefficient Y_{SX} indeed varies with the growth rate.

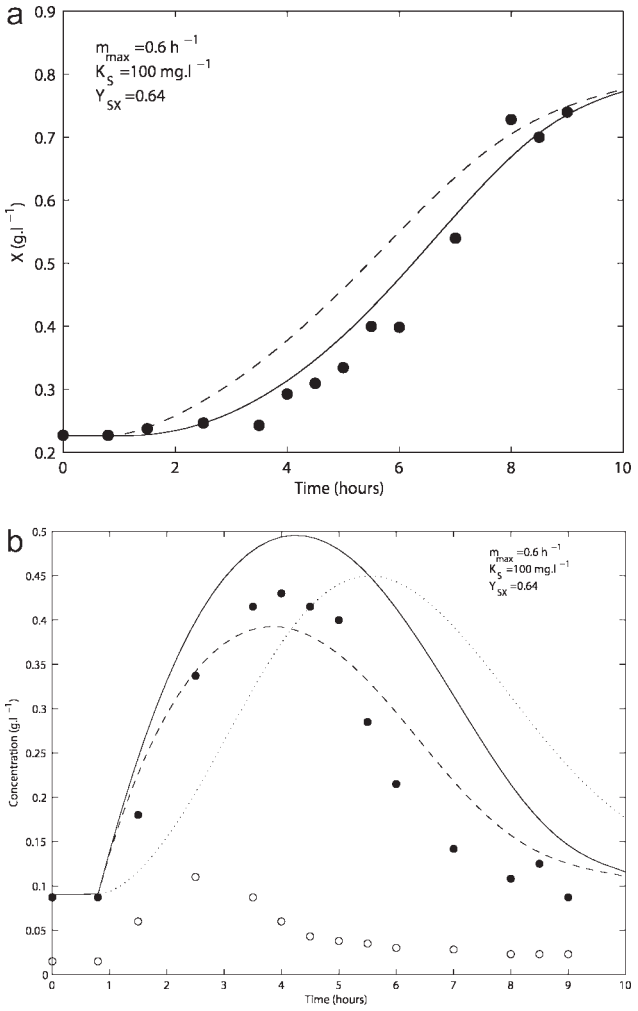

Figure 5. Response of a continuous culture of activated sludge to a change in the glucose concentration at the inlet from 400 mg.l^{-1} to 1450 mg.l^{-1} at constant dilution rate D=0.244 h^{-1}. Biomass concentration (a), substrate concentration (b).

Dashed line: unstructured model, continuous line: structured model; symbols (●, ○: carbohydrates): experimental data from Gaudy.[50]

Discussion

The time scale of structured kinetic models is $1/\mu_{max}$. Thus, in these models, biological and physical phenomena with a characteristic time smaller than $1/\mu_{max}$ are filtered. Similarly, the associated length scale is the size of the reactor: mass balance equations are performed for the entire reactor. Consequently, the phenomena with a length scale smaller than the reactor scale, typically spatial heterogeneity of the concentration field within the reactor, are filtered. So, such models deal with the mean values of the variables resulting from a double averaging in time and space. However, the consequences of small scales "fluctuations" are considered in the modeling and grouped into static equations (comments on Y_{SX}, above). As a result, when a homogeneous reactor approach is used, the different laws and parameters of the biokinetic model indeed account for both physical and biological phenomena that occur locally. This is also true for the structured model proposed in this article, and it imposes a constraint on the hydrodynamic part of the model also. It makes sense to combine this structured model for bioreaction with a hydrodynamic model consisting of a number of interconnected perfectly mixed reactors, provided each reactor has a residence time of about $1/\mu_{max}$ or more. But the use of Eq. 5 to model the instantaneous growth rate may not be appropriate in combination with a spatially refined model for the fluid flow (as in a Computational Fluid Dynamics model). Thus, the form of the equation accounting for adaptation dynamics cannot be chosen independently from the model used to represent the hydrodynamics of the reactor.

Basically, the consumption of substrates in the liquid phase results from a mass transfer, between a liquid phase and a biotic phase. This occurs at the cell scale. The space averaging of these local mass transfer fluxes over the volume of the entire reactor leads to a sink (or source) term in the conservation Eqs. 2 and 3. For a matter of simplification, this term has been modeled as a homogenous kinetic reaction term, which it is not. So, Eq. 6 is of central importance in the model because it refers to both the transfer and the reaction. On the one hand, it reflects that the actual growth rate may be lower than the equilibrium growth rate if cells are not adapted to the environment. On the other hand, if cells face a decrease of substrate availability, it limits the growth rate to the equilibrium value, the value that would be observed at steady state in the same environment. The first situation represents a limitation in the biological phase (reaction rate is limited by the reaction in the biological phase), whereas the second situation holds for a limitation in the liquid phase (reaction rate is limited by the transport down to the cell membrane).

The structured model proposed decouples the growth rate from the concentration of the substrate in the liquid phase. This enables the correct prediction of the instantaneous growth rate. There is still a strong coupling between the growth rate and the assimilation rate, via Y_{SX} which can be constant or related to the growth rate $Y_{SX} = f(\mu)$. It can be observed in Figures 3b and 5b that the substrate concentrations are overestimated by the model, i.e., the substrate assimilation rate is always underestimated. This indicates that the calculation of the substrate uptake rate from the growth rate and Y_{SX} is not satisfactory under transient conditions. This can be explained by the fact that Pirt's law is a stationary law in the sense that it is obtained from a series of steady state experiments. Microbiologists generally agree on the fact that starvation and suboptimal levels of substrate induce non-negligible consequences on bacterial metabolism.[52] Among these, one can note the fact that bacterial cells adapt their substrate uptake capacities to the concentration fields they undergo.[53] Neubauer and coworkers[5,54] have shown that cells cultivated under substrate (glucose) limiting conditions (starvation period during 27 minutes) but regularly exposed to substrate excess (for 2 minutes) respond by an increase of their substrate uptake capacity. As a result the instantaneous uptake rate measured after the limitation relief is up to six times higher than the maximum uptake rate measured in a batch culture. This capability to exhibit an instantaneous uptake rate that was not correlated to the growth rate has also been reported by Leegwater et al.[20] They have measured that the substrate uptake rate after a pulse addition of glucose to a continuous culture is higher than what it would have been in steady state at the same dilution rate. The substrate uptake rate was a maximum and constant whatever the dilution rate for *K. aerogenes*, whereas the uptake rate was positively correlated to the dilution rate for *E. coli*. Natarajan and Srienc[55,56] have confirmed by recent techniques that growth rates and substrate uptake rates were decoupled in the range of growth rates studied. Ferenci has explained that, when exposed to limiting concentration, cells develop additional systems (i.e., others than PTS) for the transport of glucose, characterized by a high affinity for the substrate (very low K_S value).[52,53,57] The activity of the different transporters is correlated to the substrate concentration in the liquid phase. At high and low concentrations, facilitated diffusion is the preferred mechanism, whereas for intermediate concentrations, the active transport using a PTS is dominant and positively correlated to the substrate concentration.[58]

From these experimental results, it is now possible to conclude that cells can exhibit an extra assimilation capacity under transient conditions, resulting in the assimilation of a certain amount of excess substrate when exposed to a high substrate concentration step change. The actual instantaneous uptake rate of the cell population is therefore a consequence of the culture history.[54] Thus, the consumption rate of substrate, r_S, as defined in Eq. 2 is not suitable for two reasons

(1) The growth rate and the substrate uptake rate are partially decoupled under transient conditions.

(2) Y_{SX} is either constant or built on a stationary law.

From a modeling point of view, these experimental results give rise to further issues:

(1) How can the substrate uptake rate be predicted under transient conditions if it is not directly correlated to the growth rate?

(2) How can the accumulation of excess substrate be quantified?

(3) What does the extra amount of substrate become inside the cell?

This first question was addressed by Chassagnole et al.[34] in the case of a glucose uptake rate determined by the phosphotransferase system (PTS). These authors have shown that the PTS system adapts to the concentration of substrate in the liquid phase very rapidly. The time scales associated to this adaptation range from 0.29 to 85 s, which is very small compared with the time scale of growth. Thus, a valuable solution in our dynamic model (the characteristic time scale of which is $1/\mu_{max}$) is to set the instantaneous uptake rate to the value computed from the concentrations in the liquid phase.

$$\phi_S = \frac{r_S}{X} = \frac{1}{Y_{SX}}\mu^0 \tag{16}$$

Figure 6a, b represent the results obtained when the substrate consumption rate, r_S, is computed from Eq. 16. The same set of parameters as in Figure 5a, b has been used. The estimation of the biomass is slightly better in this case but the use of Eq. 16 greatly enhances the prediction of the substrate accumulation especially just after the perturbation and up to $t = 4$ h. Under transient conditions, the choice of Eq. 16 seems consistent and should be valid as long as the PTS is the principal mechanism responsible for the substrate uptake. If the latter hypothesis is not valid, then Eq. 16 will still lead to an underestimation of the actual uptake rate since other transporters should be considered in the model for substrate assimilation.

Considering that the growth rate is correctly predicted by our model, the accumulation of excess substrate at any time may be related to the difference between the instantaneous uptake rate (Eq. 16), and the overall utilization rate that would be observed in steady state at the same growth rate. The latter can be evaluated as $1/Y_{SX}\ \mu^a$. This overall rate encompasses utilization of substrate for growth, storage and also maintenance.

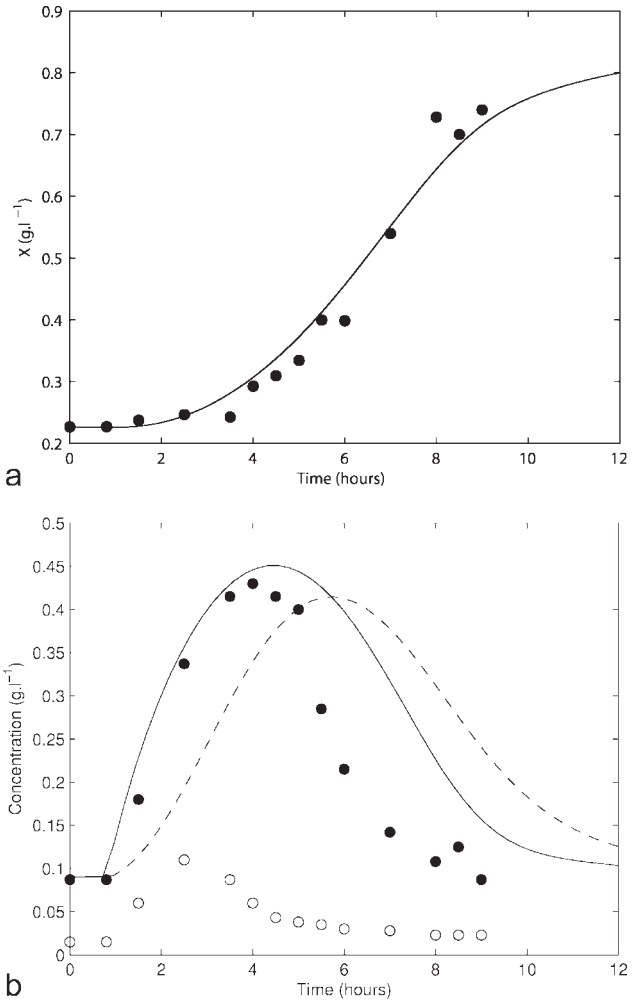

Figure 6. Response of a continuous culture of activated sludge to a change in the glucose concentration at the inlet from 400 mg.l^{-1} to 1450 mg.l^{-1} at a constant dilution rate D=0.244 h^{-1}. Biomass concentration (a), substrate concentration (b).

Continuous line: structured model (substrate uptake rate computed from Eq. 16); dashed line: internal variable; ●: experimental data from Gaudy.[50]

$$\phi_{S,acc} = \frac{1}{Y_{SX}}\mu^0 - \frac{1}{Y_{SX}}\mu^a \tag{17}$$

Indeed, the extra amount of substrate is not supposed to accumulate in this form over a long period of time as it will be converted into storage product[19] or dissimilated (overflow metabolism).[32] Its fate within the cell is highly dependent on the biological phenomena considered in the metabolic model. Although attempting to model storage and growth processes simultaneously for activated sludge systems, Vanrolleghem et al.[17] have proposed modeling the dynamics of growth and storage using a first order time delay function $(1 - e^{-t/\tau})$. Sin et al.[19] have stipulated that the ratio between the substrate uptake rate and the substrate flux diverted to storage is constant. The authors report that this ratio is constant under feast phase but also point out that its value is influenced by many factors among which the history of biomass and the feast/famine ratio are present.

If the proposals made in Eqs. 16 and 17 are meaningful:

(1) The growth rate is correctly predicted without any time delay function.

(2) The substrate uptake rate is obtained from Eq. 16.

(3) The ratio of substrate uptake/storage is not necessarily constant.

If there is no limitation due to oxygen, i.e., the excess amount of substrate is not dissimilated, the specific rate of additional formation of storage product under transient conditions can be written as:

$$\frac{r_{STO}}{X} = v_{STO}.\phi_{S,acc} \tag{18}$$

The ratio of substrate storage against substrate taken up under transient conditions, computed from Eqs. 16 and 18, is represented on Figure 7. The value of v_{STO} is set to one for simplicity. This curve reflects the changes in the storage/uptake ratio by comparison with a steady state situation. A value higher than zero corresponds to an increase in the internal substrate concentration leading to faster storage. On the opposite, a value smaller than one means that rate of storage is less than what it would be at a steady state.

In the modeling approach of Sin et al. it has been assumed that the internal substrate concentration was at steady state. This assumption has been further discussed, and it has been found to be incorrect at the time of the pulse addition and just after the depletion of the substrate in the medium.[19] As a consequence the storage/uptake ratio is also expected to vary at these particular moments. Indeed, this trend can be observed in Figure 7. Furthermore, it seems reasonable to consider that the ratio substrate storage/uptake is almost constant between 3 and 8 h, equal to the value at a steady state, since the variation of the ratio is close to zero during that period of time.

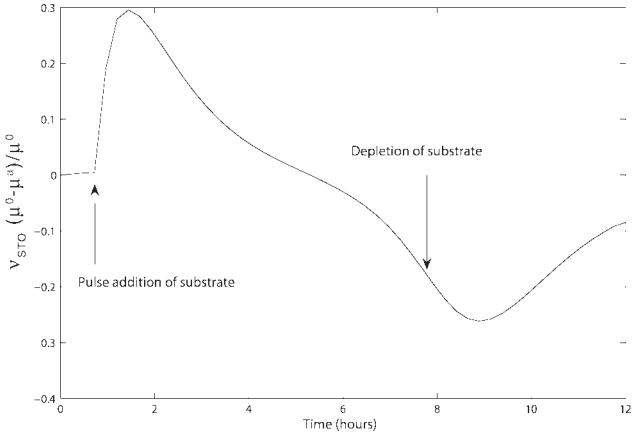

Figure 7. Variation in the ratio of storage product to substrate taken up under transient conditions by comparison with the value at a steady state after pulse addition of substrate at t = 1 h.

The experimental data reported in Figure 5b reveal that some overflow metabolism has occurred leading to the excretion of carbohydrates in the liquid. In the metabolic model proposed by Xu et al. for *E. coli*, this overflow is related to the limited oxidative capacity of the cells. So to account for this phenomenon, this model should be complemented with another internal variable that would represent the oxidative capacity of the population. This would allow the extra amount of substrate to be diverted either to storage or dissimilation, depending on the oxidative capacity.

Future works on the coupling of bioreactions and hydrodynamics will have to address the issue of calculating local instantaneous uptake rates at the cell scale. Indeed, as the spatial resolution increases (Computational Fluid Dynamic based model of the bioreactor), the characteristic time scale of the physical phenomena considered in the global model decreases. Thus, one has to know about the local and instantaneous values of the mass fluxes exchanged between the environment and the cells. Lapin et al.[8] have shown that the prediction was possible if the main system for glucose uptake is the PTS.[59] However, there is still some work to be done to correlate the substrate uptake rate to the growth rate. Furthermore, the calculation of the substrate uptake rate when other transporters are also involved is not an easy task. The works of Ferenci[52,53,57] suggests that it cannot be done with reference to the usual biological characteristics issued from the steady state observations.

Conclusions

The structured model has been developed from theoretical considerations and includes some experimentally based knowledge on the dynamic behavior of biological systems. It has been tested against three sets of experimental data without any adjustment. In this regard, the dynamic aspects of the transient response are qualitatively reproduced by the proposed model. This constitutes a clear improvement in comparison to the standard unstructured model, with a moderate increase in the model complexity. The comparison with various data sets suggests that the adaptation is controlled by a single time constant. So, provided that the biological constants K_S and μ_{max} are known a priori, the proposed structured model only requires the identification of one additional constant τ. The value $\tau = 1/(0.8\ \mu_{max})$ is proposed as an initial guess. The structured model is formulated in terms of extensive variables, and it is therefore easy to couple with any hydrodynamic model provided the time scale of the latter remains greater than $1/\mu_{max}$.

Notation

Roman

a = gas-liquid interfacial area ($m^2\ m^{-3}$)
e = intracellular compound concentration (AU g_X^{-1})
e^* = intracellular compound concentration at equilibrium (AU g_X^{-1})
k_e = half saturation constant (AU g_X^{-1})
m = maintenance coefficient
q = adaptation rate (h^{-1})
r = volumetric reaction rate ($g\ L^{-1}\ h^{-1}$)
t = time (h)
w = weighted average of substrate concentrations ($mg\ L^{-1}$)
A_e = specific adaptation rate ($g_e\ g_X^{-1}\ h^{-1}\ m^{-3}$)
D = dilution rate of the reactor (h^{-1})
K_{eq} = equilibrium constant [e^*/S]
K_LA = oxygen mass transfer rate (h^{-1})
K_S = half saturation constant ($g\ L^{-1}$)
C_{O_2} = oxygen concentration ($g\ L^{-1}$)
C_{O_2} = oxygen saturation concentration ($g\ L^{-1}$)
COD = Chemical Oxygen Demand ($g\ L^{-1}$)
Q = volumetric flow rate ($m^3\ h^{-1}$)
S = carbon substrate concentration ($g\ L^{-1}$)
V = volume of the reactor (m^3)
X = biomass concentration ($g\ L^{-1}$)
Y_{SX} = yield coefficient of biomass on substrate ($g_X\ g_S^{-1}$)
Y_{OX} = yield coefficient of biomass on oxygen ($g_X\ g_O^{-1}$)

Greek letters

α = adaptation rate (h^{-1})
ϕ_S = substrate uptake rate ($g_S\ g_X^{-1}\ h^{-1}$)
$\phi_{S,acc}$ = substrate accumulation rate ($g_S\ g_X^{-1}\ h^{-1}$)
μ = growth rate (h^{-1})
μ^0 = growth rate at equilibrium (h^{-1})
μ^b = biological growth rate (structured model) (h^{-1})
μ^a = actual growth rate (structured model) (h^{-1})
v_{STO} = stoechiometric coefficient ($g_{STO}\ g_S^{-1}$)
τ_+ = time constant, used when $e < e^*$ (h)
τ_- = time constant, used when $e > e^*$ (h)

Subscript

in = value at the inlet
S = substrate
STO = storage product

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