Review of procaryote metabolism in view of modeling microbial adaptation from fast growth to starvation conditions

B. Lavallée, P. Lessard, and P.A. Vanrolleghem

Abstract: More than 50 years ago, Monod (1949) proposed the application of the Michaelis–Menten relation describing enzyme kinetics to a culture of microorganisms. For the purpose of simplification, the mathematical relation proposed by Monod (1949) reduced the entire cell to a single enzyme genetically expressed in a single intensity. However, cell metabolism is based on a large number of biochemical reactions. This paper reviews the literature to identify the controlling factors of cell metabolism and the regulation of specific activity of the cell. The literature review was designed to highlight which regulation mechanisms induce a growth-rate variation so that they can be expressed mathematically. The study of these processes will focus on modeling the specific activity variation. The review is limited to heterotrophic procaryote organisms under aerobic conditions.

Key words: modeling, metabolism, regulation, growth rate, cell death, maintenance, activated sludge.

Résumé : Il y a plus de 50 ans, Monod (1949) a proposé d'appliquer l'équation de Michaelis-Menten décrivant la cinétique de l'enzyme à une culture de microorganismes. À des fins de simplification, la relation mathématique proposée par Monod (1949) réduit la cellule entière en un seul enzyme génétiquement exprimé à une seule intensité. Toutefois, le métabolisme de la cellule est basé sur un grand nombre de réactions biochimiques. Le présent article revoit la littérature afin d'identifier les facteurs contrôlant le métabolisme de la cellule et la régulation de l'activité spécifique de la cellule. La revue de la littérature a été conçue pour souligner les mécanismes de régulation qui induisent une variation du taux de croissance afin de les exprimer mathématiquement. L'étude de ces procédés portera sur la modélisation de la variation de l'activité spécifique. La revue est limitée aux organismes procaryotes hétérotrophes sous des conditions aérobies.

Mots clés : modélisation, métabolisme, régulation, taux de croissance, mort cellulaire, maintenance, boues activées.

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1. Introduction

Cell growth modeling is used mainly in wastewater treatment, more specifically for activated sludge processes. Several models, all of which are based on Monod kinetics, have been proposed to represent and predict the dynamic response of these processes (Lessard and Beck 1991; Henze et al. 2000). Current models assume that the cells, or the biomass, have a single level of expression or specific activity. The experimentally determined parameters are thus inherently dependent on the operating conditions and the configuration of the system under study (Henze et al. 2000).

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However, cell metabolism is based on many biochemical reactions. All these reactions occur in ordered sequences and are regulated by different inducers and inhibitors. There are thousands of different enzymes and several hundred of them may be involved in these reactions (Bailey and Ollis 1986).

As the cell has a variable specific activity level, any variation in process operating conditions or any modification to the system will cause a deviation in model response. For instance, it is well known that μ_{Hmax}, the maximal growth rate of heterotrophic biomass, will change according to culture conditions (Daigger and Grady 1982*a*). Also, k_{STO}, the rate of storage in activated sludge model of International Water Association (ASM3) is not universal, and the value of this constant changes with environmental conditions (Hanada et al. 2001).

An engineer striving to optimize a system configuration or wishing to modify a given system has therefore little chance to predict the system response to modifications, to define the optimal configuration, or to determine the corrective actions that would be best. Actually, engineers use standard design rules and their knowledge to design wastewater treatment plants (Gernaey et al. 2004). Models are being used more and more often, but in such cases, ASMs can be of only some assistance in optimization of design and operation because they do not take into account the metabolic adjustment of the active biomass and subsequent variation of the parameters. Wild et al. (1994)

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showed the value of this problem for denitrification in the activated sludge process. Variation of the volume ratio or of the anaerobic:aerobic:anoxic time ratio changes the induction time of particular enzymatic chains. Thus, this changes the level of specific activity of the biomass and the dependent parameters. Schmid (2002) showed this phenomenon with resonance induction. Therefore, to perform the optimization process with ASMs, several experiments must be conducted, as done by Schmid (2002). Liu et al. (1999) proposed some modifications to ASM1 to optimize the aerobic–anoxic period in a denitrification process and to minimize the experimental part. These authors proposed the inclusion in ASM1 of some cybernetic functions to model the diauxic growth on O_2 and NO_3. Similar analysis should be done to optimize the sequencing batch reactor (SBR) process. In this case, a number of possibilities should be analyzed. van Loosdrecht and co-workers (Smolders et al. 1994, 1995*a*, 1995*b*; Kuba et al. 1997) published several papers on SBR, but in most of these publications a 2:4 anaerobic:anoxic period ratio was used. Kuba et al. (1997) performed a validation of the "Technical University Delft Phosphorus" model on different cycle lengths but it was calibrated on experiments with similar "total induction time". Therefore, recalibration of the parameters was not required. This work aims to model the variations in rates under transient conditions or under different induction times. Thus, it would help an engineer to choose the optimal ratio of volumes or cycle lengths (induction time) and the optimal process configuration. A new or improved model would help in understanding the transient behaviour of the activated sludge process and in optimizing the design and operation of the process.

Also, nucleic acid probes are used more and more often in studies of wastewater treatment processes and microbial ecology (Amann and Ludwig 2000; Wilderer et al. 2002). So, quantification of active cells with probes will require models with further refinement in the description of active biomass, as variation of specific activity is taken into account. Moreover, ribosomal ribonucleic acid (rRNA) theory does not explain all transient behaviour of active cells (Daigger and Grady 1982*b*). According to biochemistry literature, the mechanisms describing the growth process seem to be well understood, but the dynamics of the whole processe are not yet well defined (Cangelosi and Brabant 1997; Muttray et al. 2001). Therefore, the development of more realistic mathematical models is necessary and appropriate. Regulation of cell growth rate should be integrated into mathematical models to predict biomass behaviour after system modifications.

The objective of this paper is thus to review the literature and from it deduce a conceptual model that mimics the variation of the specific activity or the regulation of growth of active biomass. With such a model, it should be possible to access the intrinsic value of parameters (parameters independent of the physiological state of the organism (Grady et al. 1996)) defining the growth rate of the biomass on a given substrate. Therefore, with the same set of parameters, it would then be possible to predict the response of continuous systems or semi-continuous systems and batch processes, which is not possible with ASMs.

In this paper, the substrate metabolism will be studied following the main metabolic pathways; information from the literature review will be used to probe the cell metabolism more deeply. This study focuses on regulation of the processes at the transcription level, i.e., regulation of deoxyribonucleic acid (DNA) code expression and regulation of the cell's specific activity. In the first part of the paper, the substrate uptake and its use are studied. The review will discuss first the substrate uptake rate (Sect. 2) and then bifurcations in metabolism, such as the storage products and soluble microbial products (SMPs) or exopolymeric substances (EPSs) (Sect. 3). In the second part of the paper (Sect. 4), the central metabolism is summarized as a growth process and studied through the protein synthesis system (PSS), which is a major component of the regulation of the growth process (Zhang et al. 2002). A simplified representation of the PSS is presented to model the regulation of the growth process. In the third part of the literature review (Sect. 5), phenomena that have an influence on the evaluation of yield or growth rate, such as cell death and maintenance, are studied. Finally, some extra-cellular mechanisms, such as the evolution of the population of microorganisms in activated sludge, or predation, are discussed (Sect. 6). Therefore, several processes are studied to get an overview of what occurs in activated sludge in terms of adaptations or transient phenomena. A companion paper presents the model mathematically with validation results (Lavallée et al. 2005).

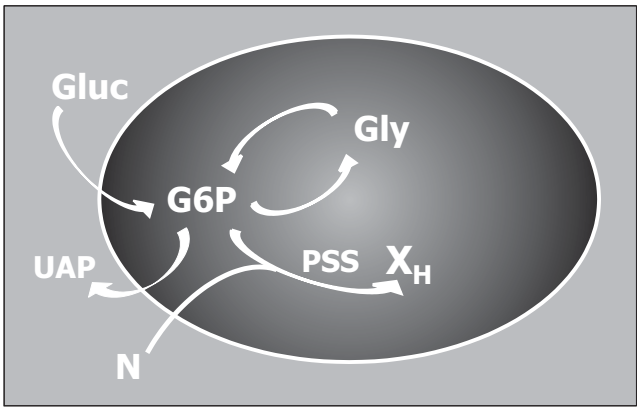
1.1. A quick overview of the main metabolic pathways

The cell's ability to multiply depends mainly on its capacity to assimilate a given substrate and to metabolize it into cell constituents. The regulation of various metabolic pathways represents the ability of the cell to adapt to its environment. The regulation mechanisms of these processes must be reviewed to model them properly. The literature review is limited to procaryote heterotrophic organisms exposed to aerobic conditions.

In short, it could be said that the substrate uptake rate is regulated by the substrate transporter density in the cell membrane, which increases with the substrate concentration (Ferenci 1999). It seems that this regulation process changes the affinity constant of the biomass for the substrate. Following its uptake, the substrate could be used for growth, but several bifurcations exist in the cell metabolism. The two main bifurcations studied in carbon metabolism are the formation of storage products and the production of SMPs. Storage products are mainly polyhydroxybutyrate (PHB) or glycogen (Dawes and Senior 1973). Their rate of formation and degradation is regulated by different metabolites as well as at the transcription level (Preiss 1996; Kessler and Witholt 2001). Production of SMPs is not yet well understood, and the following discussion attempts to give an overview of the state of the knowledge about SMP production.

Regulation of the growth process is associated mainly with regulation of the PSS (Zhang et al. 2002). The transcription

Fig. 1. Schematic representation of main metabolic pathways. G6P, glucose 6-phosphate; Gly, glycogen; PSS, protein synthesis system; X_H, heterotrophic organisms; N, nutrients; UAP, utilization-associated products; Gluc, glucose.



and the translation are two key steps in protein synthesis that are regulated by the energy level of the cell (Zhang et al. 2002).

A simplified schematic of these mechanisms is presented in Fig. 1. In this figure, glucose (Gluc) is taken up by the cell and used to form an intracellular substrate (glucose 6-phosphate (G6P)). The latter could be used for growth (X_H) or formation of utilization-associated products (UAPs) or glycogen (Gly). A similar representation was adopted by Dircks et al. (2001). Beun et al. (2000) used a similar representation for cells growing on acetate. According to these authors, acetate is used to form pyruvate as endogenous substrate, and the latter forms PHB rather than glycogen as a storage product. Also, as indicated in Fig. 1, nutrients (N) are required for growth. The growth process is regulated by the state of the PSS. The PSS is discussed below.

The more detailed discussion that follows emphasizes regulation dynamics. The focus is on regulation at the transcription level, i.e., the expression of enzymes associated with the particular process under discussion.

2. Substrate uptake

Because the cell prioritizes consumption over growth (van Loosdrecht et al. 1997) and this process is inducible (Ferenci 1999), modeling the substrate consumption rate becomes important. This impacts the storage processes and SMP formation. Moreover, it is possible to observe in experiments with activated sludge that the specific substrate consumption rate varies according to culture conditions (Beun et al. 2000; van Aalst-van Leeuwen et al. 1997; Sin 2004). Sin (2004) clearly showed that the substrate uptake rate increases with the initial substrate to biomass ratio (S_0/X_0).

Certain authors have demonstrated that cells adapt the transport enzyme density in the membrane according to environmental conditions (Ferenci 1999). Also according to Ferenci (1999), the level of expression of various transport enzymes of glucose is regulated by the substrate concentration. Three types of porins are located on the external membrane and may affect its permeability. Three types of transport enzymes are located on the cytoplasmic membrane. Apparently, the substrate uptake rate is dependent on the induction intensity or, in other words, on the porin and enzyme concentration in the cell wall. At low substrate concentrations, a transport enzyme system with a high affinity would be induced at a higher intensity than a transport system with a low affinity, which is favoured at high glucose concentrations. Ferenci (1999) proposed mathematical equations to model the substrate uptake rate.

Therefore , when trying to model a transient culture, one would perhaps have to model the regulation of the substrate uptake rate at the induction level. According to Fig. 1, the induction intensity of the transport enzyme(s) of the exogenous substrate is regulated by the concentration of this substrate.

2.1. Variation of the affinity constant

As already mentioned, according to Ferenci (1999), the intensity of expression of various transport enzymes varies with substrate concentration. It also seems that these enzymes show different affinity constants (K_S). Kovárová-Kovar and Egli (1998) established a relationship between the affinity constant and the growth rate of *Escherichia coli* on glucose. A high growth rate and low glucose concentration resulted in the derepression of a high-affinity transport system. High substrate concentrations favoured the induction of a low-affinity system (Ferenci 1999).

It seems that K_S is not a constant for mixed cultures, but rather a variable that depends on the species selected and their relative population size, the metabolic state of the species responsible for substrate degradation, and growth rates (Kovárová-Kovar and Egli 1998). Moreover, based on the competition principle, we know that rapid-growth bacteria populations show a higher affinity constant than slow-growth species (Stumm-Zollinger and Harris 1971). Modifications in the population structure of the biomass may therefore cause variations in the affinity constant.

Moreover, some chemical phenomenon, such as mass-transfer resistance in large flocs, may affect the assessment of the affinity constant (Characklis 1978; Shieh 1980; Lau et al. 1984; Chu et al. 2003). These phenomena must be taken into account in the calibration procedure of a model.

One can see that the cell itself adapts to the environment, but at the same time, the population structure and floc size may affect the affinity constant value. Therefore, the formulation of a model to model affinity constant variation may be complex.

3. Bifurcations in the use of metabolites by the cells

"Micro-organisms capable of rapidly storing and consuming substrate in a more balanced manner have a competitive advantage over organisms that cannot do it" (van Loosdrecht et al. 1997). During a starvation period, cells with this competitive advantage increase their chance of survival. In fact, the storage of

reserves becomes paramount during batch or semi-continuous system (RBS) cultures.

The substrate that is taken up could be used for growth as well as for storage. The storage process is well depicted in some metabolic models (Henze et al. 2000; Smolders et al. 1994), but these models do not take into account the regulation of the process at the transcription level. Sin (2004) showed that the rates of these processes could vary with the original substrate to biomass ratio (S_0/X_0) in batch experiments. Thus, the following discussion focuses on the induction mechanisms to highlight how transcription regulation could change the specific rates.

A similar discussion will be presented on other bifurcations of the cell's metabolism, such as SMP formation and EPS production.

3.1. Accumulation of storage products

Some of the models proposed in the literature tend to model the kinetics of biomass growth, not on external substrate but rather on internal substrate or on endogenous reserves (Krishna and van Loosdrecht 1999; Henze et al. 2000). However, these models do not take into account the variation of the specific rate of reserve synthesis. Certain observations showed that the specific polyhydroxyalkanoate (PHA) production rate varies with operating and environmental conditions (Hanada et al. 2001).

Metabolic pathways used by the cell to produce glycogens or PHB are relatively well known (Dawes and Senior 1973). Glycogens are formed from glucose 6-phosphate through a sequence operated by four enzymes (Dawes and Senior 1973; Preiss 1996). The formation of PHB starts with acetyl-CoA and follows a sequence operated by three enzymes (Dawes and Senior 1973; Kessler and Witholt 2001). According to the model presented by Dircks et al. (2001), it would be possible to model glycogen synthesis or degradation using a single reaction. According to Beun et al. (2000), this would also be true for PHB.

However, the synthesis regulation mechanisms for PHB are not well known. Regulation is carried out at the genetic transcription level and at the enzymatic level (Kessler and Witholt 2001). Kessler and Witholt (2001) proposed a biochemical model in which genetic expression of PHB synthesis and degradation enzymes is inhibited by absence of substrate for PHB production. The nicotinamide adenine dinucleotide phosphate, reduced form, to nicotinamide adenine dinucleotide phosphate, oxidized form (NAD(P)H/NAD(P)) load regulates the enzyme activity and thus the formation and degradation of PHB (Dawes and Senior 1973; Kessler and Witholt 2001). Also, depolymerization of PHB appears to be inhibited in the presence of a soluble substrate favouring rapid growth (Kessler and Witholt 2001). However, certain observations seem to indicate that degradation of PHB occurs at the same time as its synthesis (Kessler and Witholt 2001).

Glycogen production is regulated by genetic expression and enzyme activity (Preiss 1996). A high-energy charge (the adenylate forms ratio) removes the inhibition of the enzymes responsible for glycogen synthesis. The cell appears to regulate its metabolic capacity to convert the carbon substrate into glycogen in response to the substrate availability (Preiss 1996). This regulation occurs at the level of gene expression through cyclic adenosine monophosphate and guanosine tetraphosphate (ppGpp) (Preiss 1996). Also, the expression of enzymes responsible for the synthesis and degradation of glycogen is regulated by the same operons (Preiss 1996). Therefore, one can assume that their expression is proportional.

One can also assume that the expression of enzymes involved in reserve synthesis and degradation would be proportional and regulated by the G6P concentration (the operons inductor) in the cell (Fig. 1).

3.2. Production of soluble microbial products

In addition to storage phenomena, metabolite release processes have been observed by several authors (Grady et al. 1972; Huang and Cheng 1987; Hao and Lau 1988). It seems that most of the soluble organic matter in the effluent of a wastewater treatment plant is SMP (Barker and Stuckey 1999). Solid microbial products include a large variety of products, such as humic and fulvic acids, polysaccharides, proteins, nucleic acids, various organic acids, antibiotics, steroids, cell structural components, and metabolic products. The molecular weights of these products can vary from <500 to $>50\,000$ Da (Barker and Stuckey 1999).

According to the literature review by Barker and Stuckey (1999), SMPs are composed of all soluble products leaving the biological system that were not present in the influent. A part of these products seems to be biodegradable and of low molecular weight. These products are associated with growth (UAPs) and accumulation of intermediate metabolites. The UAPs are generally defined as metabolic products derived from the substrate (Barker and Stuckey 1999).

Hao and Lau (1988) proposed an exponential relationship of the growth rate to describe the specific production rate of UAP, especially at high dilution rates. According to these authors, a limitation in nutrients or in oxygen tends to favour UAP production. Also according to them, biomass capable of producing PHB and extra-cellular polymer reserves produces less UAP.

Chang and Rittmann (1989) proposed a Luedeking–Piret type of relationship to describe UAP production. Others (Furumai and Rittmann 1992; Lu et al. 2001) have used this relationship in several other models to describe SMP production. Models presented by some of these authors correspond well to experimental results (Lu et al. 2001), but do not shed light on UAP or SMP metabolism.

Because the UAP production rate and the biomass growth rate are proportional to the intracellular metabolite concentration, we could represent this model graphically (Fig. 1). The UAP production could thus follow an exponential relationship in relation to growth rate, as proposed by Hao and Lau (1988). Modeling UAP production during nutrient or oxygen shortages and assessing the effect of storage processes is possible with this representation.

Another part of the SMP consists of molecules with high molecular weights and is associated with endogenous respira-

tion or cellular lysis of the biomass (BAPs). These high molecular weight SMPs seem to be mainly humic and to have toxic effects on biomass (De Souza Melo 1984). Other BAPs seem to form at the end of the exponential growth period, such as certain EPSs (Davies 1999), or during toxic or thermal shocks (Barker and Stuckey 1999). Biomass-associated products include several molecules, and additional research is required to identify all their production mechanisms (Barker and Stuckey 1999).

3.2.1. Aerobic fermentation

Several authors (Akesson et al. 1999; Aon and Cortassa 2001; Chang et al. 1999; Doelle et al. 1982; Han et al. 1992; Majewski and Domach 1990) have reported aerobic fermentation processes that can be associated with UAPs . However, the inclusion of fermentation products in UAPs remains a topic of discussion (Barker and Stuckey 1999).

It seems that above a critical substrate uptake rate, aerobic respiration can limit growth rate (Han et al. 1992; Majewski and Domach 1990; Akesson et al. 1999). Moreover, in such circumstances, the aerobic respiration should be downregulated, and this can further limit the growth rate (Doelle et al. 1982). Different hypotheses have been proposed to explain this phenomenon. Han et al. (1992) propose a limited capacity in the tricarboxilic cycle (TCA) cycle. According to Chang et al. (1999), when the respiration chain is rate limiting, the NAD(P)H/NAD(P) ratio increases, and thus the TCA enzymes are inhibited. However, these authors also showed that aerobic fermentation is due to an oversupply of pyruvate, and a mutant capable of PHB storage could maintain a normal growth rate. Aon and Cortassa (2001) showed that nitrogen-related anabolic fluxes would determine when ethanol fermentation is triggered. Han et al. (2002), using the concentration of the intracellular metabolites, showed that there is no bottleneck and proposed that the decline in μ is associated with a specific soluble microbial product, which is induced by the population density. This specific microbial product is usually called a quorum sensing signal. No clear trend was found in the literature to explain the aerobic fermentation process.

Thus, limitation of the growth rate by aerobic respiration could be introduced into the model at a later stage, when this process is better understood.

3.3. Production of exopolymeric substances

Exopolymeric substances, or exopolysaccharides, are a particular type of SMPs. The literature focuses on them because they represent the main biofilm "glue" or matrix and their production kinetics seems different than that of other SMPs (Characklis 1990).

Alginate is an EPS-type substance produced by *Pseudomonas*, among others, and it is one of the main components of the glycocalyx, or bacterial biofilm (Davies 1999). It does not seem that EPSs build food reserves, as most bacteria cannot degrade the EPSs they produce (De Vuyst et al. 2001). However, Sutherland (2001) states that several species have enzymes capable of depolymerizing EPSs. These enzymes would be intracellular, but cell lysis could help them attack EPSs.

It appears that EPS production is encouraged by the same conditions favouring PHB or glycogen synthesis, and it seems to compete for glucose 6-phosphate or glucose 1-phosphate (De Vuyst et al. 2001; Sutherland 2001; Ramos et al. 2001).

There is relatively little information on factors triggering EPS production in the environment, as the production level is generally not detectable (Davies 1999).

Davies (1999) speculates that the triggering factor in EPS production is a communication signal of the "quorum detection" type. The signal comprising a homoserine lactone type of molecule seems to control several genes, including production of EPSs and a regulon triggering the stationary phase. This author's hypothesis seems to be in accord with the fact that EPS production is inversely proportional to the growth rate.

In short, EPSs do not build food reserves but can be hydrolyzed by enzymes released during lysis. The production level is generally undetectable in the environment (Davies 1999). Production modeling of EPS would thus be required only when modeling biofilm or floc formation.

3.4. Conclusions

A schematic representation of the main metabolic pathways in the proposed model is shown in Fig. 1. A similar representation was proposed initially by Dircks et al. (2001). In Fig. 1, exogenous substrate is taken up by the cell to form an intracellular substrate. The induction intensity of transport enzyme is regulated by the concentration of the exogenous substrate. The cell can grow on the endogenous substrate, make stored products, or produce UAPs. The proposed model includes proportional expression of enzymes for synthesis and degradation of reserve products, and the expression level is regulated by the endogenous substrate concentration in the cell. The UAP production rate is proportional to the concentration of endogenous metabolites. With this view, the UAP production rate can be modeled during a nutrient or oxygen limitation, and the effect of the storage processes can be assessed. Therefore, UAP formation would be associated with the utilization of endogenous substrate, which slightly modifies the definition of UAPs and BAPs. Usually it is assumed that UAP are produced at a rate proportional to the rate of substrate utilization and BAP are produced at a rate proportional to the concentration of biomass. According to this new definition, the UAP production rate would be proportional to the concentration of intracellular substrate. Thus, it would be strongly dependent on the substrate consumption rate, but with this definition the UAPs could still be produced after the depletion of the exogenous substrate. This new definition could decrease the frequency of using the Luedeking–Piret type equations, as UAP formation and the synthesis of some other SMPs (produced at the end of the exponential growth) would be modeled with a single process. Therefore, BAP formation (produced during the endogenous phase) would be associated only with decay or cell-death processes.

4. Protein synthesis system

According to the literature, proteins represent the greatest part of cellular biomass (Bremer and Dennis 1996; Herbert 1976). The protein content of a suspension is sometimes used to define cellular biomass (Atlas and Bartha 1998). Some authors argue that "…exponential growth can be monitored as increase in proteins; this implies that growth regulation is closely coupled to control of ribosome synthesis…" (Zhang et al. 2002). Therefore, in this literature review, the PSS has been subjected to a comprehensive study to define the major determinants regulating growth rate.

Several observations indicate a correlation between the magnitude of the PSS (or the rRNA) and the cell growth rate (Bremer and Dennis 1996). Correlation between the rRNA/DNA ratio and growth rate has been clearly demonstrated for various continuous cultures with a high growth rate. This correlation is no longer valid when the growth rate decreases, which is characteristic of activated sludge (Bremer and Dennis 1996; Muttray et al. 2001; Cangelosi and Brabant 1997).

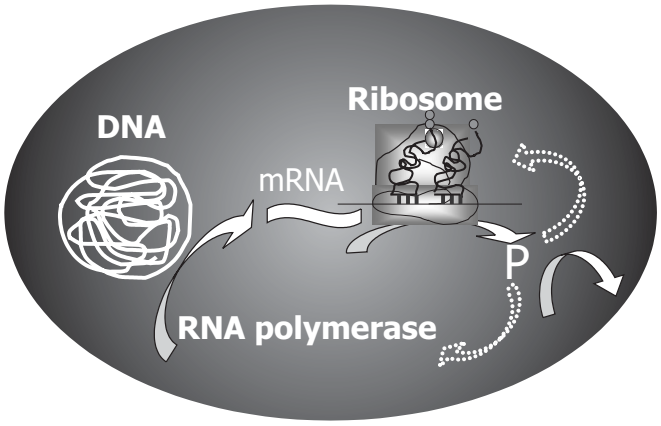
The rRNA molecule is quite stable and and does not give a good indication of the growth rate under transient conditions (Cangelosi and Brabant 1997; Muttray et al. 2001). Like the rRNA, the transfer RNA (tRNA) molecule is stable (Björk 1996), and therefore it does not provide additional information on the variation in growth rate either (Bremer and Dennis 1996; Davis et al. 1986). Pre-rRNA (a product of transcription) is a better alternative than rRNA because it has a relatively short half-life and it seems to give a better indication of the cell metabolic state during short-length transients (Cangelosi and Brabant 1997; Muttray et al. 2001; Oerther et al. 2002).

The messenger RNA (mRNA) molecule has a short time constant, and some authors argue that this component or transcription itself could play a role in regulating the growth rate (Roels 1982; Vanrolleghem et al. 1998; Zhang et al. 2002).

It is well known that transcription is a key step in the regulation ribosome concentration, and transcription of the ribosome gene (rrn) has been studied widely (Zhang et al. 2002). Some authors (Shepherd et al. 2001; Zhang et al. 2002) have observed that the transcription rate is limited by the RNA polymerase concentration in the cell. These authors proposed that the transcription activity could be described by a classical Michaelis–Menten kinetic and free RNA polymerase concentration. Moreover, adenosine triphosphate (ATP), guanosine triphosphate (GTP), and ppGpp regulate the initiation step of the transcription process of gene coding for ribosomal constituents (Jöres and Wagner 2003).

Simulation with mathematical models that include a component with a short half-life could fit the variation of the growth rate during short transients (Baloo and Ramkrishna 1991). The time constant of this component is several times shorter than that of rRNA. However, such a model would not be able to describe the long transient behaviour without changing the time constant of this component. Thus, some additional components are needed to model the PSS and, thus, short and long transients without changing the time constants and parameters of the model.

Fig. 2. The conceptual model of the protein synthesis system. DNA, deoxyribonucleic acid; RNA, ribonucleic acid; mRNA, messenger RNA; P, proteins.



The literature review that follows gives an overview of the PSS and its regulation with a view to finding the best way to model short and long transient behaviour of the active biomass.

4.1. The chosen conceptual model of the protein synthesis system

According to the preceding and following discussions, Fig. 2 shows the conceptual model of the PSS. In this model, three components, i.e., mRNA, rRNA, and RNA polymerase, make up the PSS. According to this view, protein is synthesized in two steps. During the first step, the RNA polymerase transcribes the DNA code to produce mRNA. In the second step, ribosomes translate the mRNA to produce proteins. The transcription and translation are mimicked by a classical Michaelis–Menten kinetic mechanism. Zhang et al. (2002) and Draper (1996) used similar equations for the kinetic description of transcription and translation initiation, respectively. Obviously, the real process is much more complex than this representation, and several steps and components are not included in the model.

4.2. Regulation of the protein elongation process

The objective of this paper is to present a model that mimics the variation of the specific growth rate of an active biomass. Thus, in the following paragraphs, some important factors in the regulation of these processes are highlighted.

4.2.1. Transcription regulation

The search for the determinants of the rate of transcription initiation remains at the frontiers of research (Record et al. 1996). Subunits of RNA polymerase or various elongation factors can alter the transcription rate of specific genes according to the metabolic state of the cell (Richardson and Greenblatt 1996). Therefore, the adequate description of the transcription rate of a specific gene is a complex task.

However, from a more general point of view, some authors have tried to find some of the most important determinants of

the overall transcription rate. For years, many authors (Gallant 1979; Jensen and Pedersen 1990; Östling et al. 1993; Nyström 1998) have stated that the "stringent response" regulates the transcription process. The stringent response is usually observed during shift-down experiments through the regulation of some energy carriers, e.g., ATP, GTP, ppGpp, in the cell. Thus, this mechanism was studied during shift-down and shift-up experiments because it exerts an overall control on the protein synthesis process.

4.2.1.1. Shift-down experiment and the stringent response

The stringent response seems to be an overall control mechanism of cell metabolism. The availability of carbon substrate and amino acids regulates this mechanism (Östling et al. 1993; Nyström 1998). When amino acids or carbon are deficient, the ppGpp synthease protein (RelA) fastens to the (vacant) active site of a ribosome and transfers phosphorus from ATP to GTP to form ppGpp (Cozzone 1981; Arbidge and Chesbro 1982; Gerdes 2000). Accumulation of ppGpp in the cell is related to the induction of the stringent response (Gallant 1979; Cozzone 1981; Stouthamer 1984; Östling et al. 1993; Nyström 1994; Gerdes 2000). According to Zhang et al. (2002), ppGpp decreases the activity of the initiation step and thus the rate of transcription. Other studies (Nomura 1999; Schneider et al. 2002) showed that the RNA polymerase activity is regulated proportionally to GTP and ATP. More specifically, some authors recently showed that GTP and ATP compete with ppGpp for the active site of RNA polymerase during the initiation step (Jöres and Wagner 2003). Several biochemical models are proposed in the literature, and for all of these initiation remains a key step in the transcription process (Jöres and Wagner 2003; Schneider et al. 2002; Zhang et al. 2002).

Thus, according to these authors, the transcription activity is limited by the RNA polymerase concentration, and the activity of this enzyme is regulated by the energy carriers (Jöres and Wagner 2003; Schneider et al. 2002; Zhang et al. 2002). So, the first effect of stringent response is to downregulate the RNA polymerase activity with the decrease of GTP and the increase of ppGpp levels.

Thus, according to the previous discussion, with the stringent response the GTP level decreases (and the ppGpp level increases), the transcription and the translation slow down, and owing to its high turnover, the mRNA level decreases. As ribosomes are more stable than mRNA, the latter decreases faster, and under these conditions ribosomes become in excess relative to the actual growth rate. The observations of Flärdh et al. (1992) showed that slow-growing cells could have more ribosomes than required. In other words, in slow-growing bacteria, ribosomes are undersaturated by mRNA, and according to Michaelis–Menten kinetics, a larger part of ribosomes is in standby. Effectively, the translation rate can be described by a kinetics that is dependent on the availability of mRNA and the ribosome subunits (Draper 1996). This phenomenon could explain the loss of linearity of the correlation between the rRNA/DNA ratio and the growth rate under slow-growth conditions, as in activated sludge.

4.2.1.2. Shift-up experiment

Some observations have shown that one of the first effects of a nutritional enrichment of a slow-growing biomass is an increase in the RNA synthesis rate (Kjeldgaard et al. 1958; Koch and Deppe 1971; Cangelosi and Brabant 1997). A nutritional enrichment leads to an increase in the ATP level of starved cells (Atkinson 1977). After a shift up, because the GTP level is in tight equilibrium with the ATP level (Borel et al. 1997), the GTP will increase with the ATP, and the activity of RNA polymerase will increase quickly with ATP and GTP availability. The variation of the ppGpp level occurs within 1 or 2 min (Molin et al. 1977; Lagosky and Chang 1980), and Koch and Deppe (1971) clearly showed the start-up of the transcription process within 2 min after a shift up. So, after a shift up, the transcription rate increases, and with the increase of mRNA level, the waiting ribosomes will translate the newly synthesized mRNA and raise the growth rate. Accordingly, some experiments have shown that transcription and RNA availability limit the process over only a short period of time after induction (Sandén et al. 2002; Vanrolleghem et al. 1998). The limitation of by transcription could explain the cell's ability to increase the growth rate quickly, as discussed by Daigger and Grady (1982). Thus, it is possible to make the hypothesis that, in addition to rRNA, mRNA is a key component in the description of the growth rate (Vanrolleghem et al. 1998). Because mRNA has a short time constant, this component could explain some of the observed short transient behaviours of active biomass.

After the end of the limitation by transcription, other processes will limit the growth rate. As discussed previously, a correlation between the rRNA/DNA ratio and the growth rate has been clearly demonstrated for various continuous cultures that are cultivated at high growth rates (Bremer and Dennis 1996). Thus, the translation process is also a key step in the description of the growth rate; this is known as the rRNA theory (Daigger and Grady 1982*b*). The following paragraph discusses the regulation of this process.

4.2.2. Translation regulation

As mentioned previously, the stringent response directly regulates the transcription through GTP. The GTP molecule is also required in several steps in the protein synthesis process (Prescott et al. 1990). According to Nomura (1999), the transformation of this nucleotide into ppGpp during the stringent response can limit the rate of the translation process. Schneider et al. (2002) showed the dependence of the translation rate on the ATP and GTP concentrations. Thus, transcription and translation are tightly co-regulated, as suggested by Jensen and Pedersen (1990).

In fast-growing cells, the fraction of ribosome subunits engaged in translation is constant, and with increasing growth rate the mRNA becomes more crowded with ribosomes (Bremer and Dennis 1996). The fact that the fraction of unengaged

ribosomes is constant under various growth rates, according to the saturation equation proposed by Draper (1996), indicates that the amount of ribosome subunits limits the protein synthesis rate. Elsewhere, Cole and Nomura (1986) stated that the translation process limits the growth rate.

According to Draper (1996), binding of the ribosome subunits to mRNA is a key step in the translation process. The observed translation rate depends on the binding constant, the concentration of ribosome subunits, and mRNA availability (Draper 1996; de Smit and van Duin 1990). So, in some circumstances, a low level of mRNA could limit the translation rate. However, the time constant of mRNA synthesis is small, and transcription (and thus mRNA availability) limits the process over only a short period of time after induction (Sandén et al. 2002). During exponential growth, translation seems to be the limiting factor of protein synthesis (Cole and Nomura 1986; Sand én et al. 2002). Thus, in sequence, either mRNA or rRNA could limit the rate of protein elongation and the growth rate.

4.3. Regulation of the synthesis rate of the protein synthesis system

Beyond its direct regulation of transcription and translation, it seems that GTP can also indirectly affect the size of PSSs (Jensen and Pedersen 1990).

The decrease in GTP caused by the stringent response will decreases the rRNA and mRNA synthesis rates. The stringent response results in a drop in the production of the elements that constitute the ribosomes and thus in the concentration of ribosomes in the cell. The quantity of ribosomes will thus be adjusted to the availability of precursors, and the specific elongation rate of each remaining ribosome will increase. The ppGpp synthesis rate will thus decrease, and the equilibrium state will be a compromise between the number of ribosomes and their specific synthesis rates (Jensen and Pedersen 1990).

The ribosome proteins (r-proteins) and RNA polymerase are ribosome products as well. Therefore, the regulation of their synthesis rate could also be controlled by transcription and translation. Because "the specific rate of protein synthesis changes much more slowly than the specific rate of RNA synthesis" (Koch and Deppe 1971), it appears that the translation rate limits the rate of ribosome assembly. Cole and Nomura (1986) stated that a translational feedback regulation is in fact responsible for the apparent growth-rate dependency of the synthesis of r-'proteins. The translation rate, and thus the ribosome level, could limit the rate of increase of ribosome level or the increase of growth rate (Cole and Nomura 1986). Accordingly, an increase in the ribosome concentration will show a larger relaxation time than that of RNA synthesis, as shown by Cangelosi and Brabant (1997). In this experiment, after a shift up, the level of mRNA increased quickly and reached a stable level after a few minutes, but the level of stable rRNA showed only a slow increase over 4 h. Under these conditions, after a few minutes, the ribosome concentration becomes the bottleneck of the protein synthesis.

So, for modeling purposes, we could assume that translation limits the growth rate and the increase of the growth rate after a shift up. Thus, in the model, the ribosome synthesis rate will increase with the square of the growth rate, as observed by Keener and Nomura (1996).

The rate of mRNA synthesis is dependent on the RNA polymerase level (Jöres and Wagner 2003; Schneider et al. 2002; Zhang et al. 2002), and the level of the latter is proportional to the ribosome level (Bremer and Dennis 1996). In *E. coli*, several genes of the RNA polymerase subunits are located directly downstream from the gene coding for ribosome subunit proteins (Berlyn et al. 1996). Some are co-expressed with the ribosome subunit proteins after attenuation (Steward and Linn 1991). Thus, the synthesis of RNA polymerase should be modeled in proportion to the ribosome synthesis.

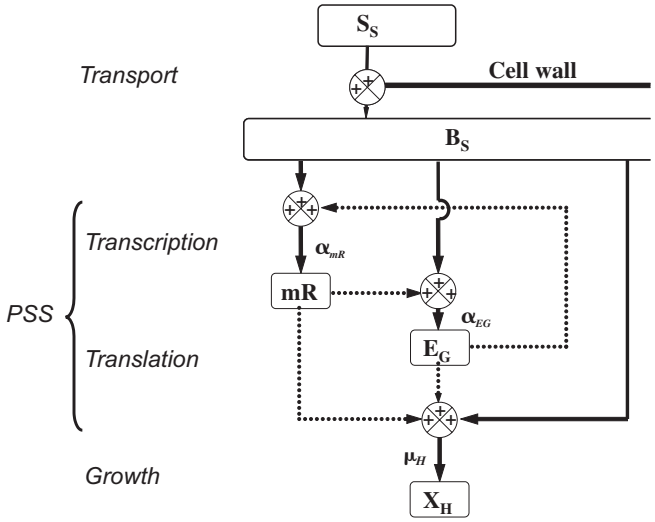
4.4. Conclusions

For modeling purposes, it appears possible to model the regulation of the cell growth rate using a mathematical representation of the PSS. Modeling of ribosome, RNA polymerase, and mRNA levels within the cell could be a first step in a proposed model. These components will describe the limitation by transcription under stringency and the limitation by translation under fast-growth conditions. Accordingly, after a shift up, the growth rate will increase quickly with mRNA and afterwards it will increase slowly with an increasing ribosome level, as observed by Kjeldgaard et al. (1958) and Cangelosi and Brabant (1997). Under slow-growing conditions, because mRNA has a high turnover, it will reduce quickly, and according to the Michaelis–Menten kinetics, the ribosomes will be in excess when compared with the actual growth rate. This seems to be in agreement with the observations of Flärdh et al. (1992).

The regulation of transcription and translation is shown in Fig. 3. In this figure, the processes are represented by bolded arrows and the process regulation by dashed arrows. The components are represented by boxes. The substrate transport process is modeled by the uptake of the external substrate (S_S) and the endogenous substrate (B_S) formation. The transcription process is modeled by the increase of the short half-life component of the PSS, mR. The RNA polymerase and the ribosomes are modeled with the growth enzyme component, E_G. The component mR has a short time constant, as does mRNA, whereas E_G has a longer one similar to rRNA. The concentration of these components in the cell (X_H) regulates the rate of the growth process (μ_H) as well as the rate of increase of their own concentrations. According to the previous discussion, the component mR limits the growth rate under stringency, and the component E_G is the bottleneck in the process during fast-growth conditions. As E_G regulates the growth rate as well as its own concentration, it increases with the square of the growth rate. After a down-shift, E_G is in excess compared with the actual growth rate.

The schematic representations shown in Figs. 2 and 3 are coherent with the current understanding presented in the literature. The ATP and the GTP are not modeled because these components have very short time constants. In the model, the energy

Fig. 3. Schematic of the protein synthesis system and growth rate regulation mechanisms. S_S, external substrate; B_S, endogenous substrate; mR, short half-life component of the PSS; α_{mR}, production rate of mR; α_{EG}, production rate of E_G; μ_H, growth rate of heterotrophic organisms; X_H, heterotrophic organisms.



level could be assumed to be proportional to the concentration of the intracellular substrates (Daigger and Grady 1982*a*). Moreover, the variation of the concentration of metabolites in the cells induces short transients (Chassagnole et al. 2002; Vanrolleghem et al. 2004) with a time constant of the same magnitude of ATP and GTP variations.

5. The decay of biomass

Several factors may affect the assessment of the growth rate or, more specifically, the decay of the biomass. Biomass maintenance and decay have a significant impact on the assessment of the growth rate (Herbert 1958; Pirt 1965). These two processes are two different interpretations of similar data and are usually described with a single parameter (Beeftink et al. 1990). However, some authors have modeled these two processes with distinct descriptions (Beeftink et al. 1990; Nicolaï et al. 1991). These were reviewed to propose a simplified metabolic representation that could lead to a mathematical definition.

5.1. Cellular death

The endogenous decay concept proposed by Herbert (1958) was used in most models and led to the concept of active and non-active biomass (Weddle and Jenkins 1971). "Discrimination between viable and dead microorganisms is a fundamental problem of microbiology" (Kaprelyants et al. 1993). So, first of all, one needs to specify the concept of cellular death and differentiate it from dormancy or a viable but nonculturable (VBNC) state. Indeed, according to some authors, certain procaryote organisms may go into dormancy and survive long starvation periods (Huisman et al. 1996). Dormancy is characterized by a low specific activity (Kaprelyants et al. 1993). Other bacteria appear to maintain their normal metabolic activity but seem incapable of multiplying. Following specific protocols, some researchers have demonstrated that it may be possible to bring the cells back to a cultivable state. These observations are the basis of the VBNC cell concept (Kaprelyants et al. 1993). A debate on the exact definition of dormancy and the VBNC state is ongoing. Nyström (2001) considers the VBNC cells as not yet dead. Nyström (2001) argues that the loss in activity of cells is the result of stochastic deterioration of proteins and DNA by attacks from reactive oxygen species. Loss of culturability can be counteracted by omitting oxygen in the starved culture. According to this author, however, the apparent resuscitation of VBNC cells is the result of growth of a small fraction of already cultivable cells.

Alternatively, according to the concept proposed by Yarmolinsky (1995), toxin–antitoxin (TA) couples would be expressed continuously during growth. The toxin would have a longer half-life than the antitoxin. Thus, in the absence of gene expression, a decrease in antitoxin concentration would leave the toxin free to act. Also, Aizenman et al. (1996) identified a TA couple called mazEF. Experimentation by these authors showed that the stringent response decreases the gene expression of the mazEF TA couple. Under experimental conditions and after abrupt increases in ppGpp (and thus an abrupt drop of GTP), only 15% of the cells survived. Moreover, the mazEF gene seems to be present in most procaryotes (Gerdes 2000).

Based on this metabolic model and because the mRNA transcription rate depends on the stringent response, the active toxin concentration in the cell should depend on the growth rate. Thus, cellular death would be regulated inversely to the growth rate, as stated by Mikkola and Kurland (1991).

However, the target of the toxin MazF is not well known, and thus the objective of this mechanism is poorly understood. A debate on the real effect of this toxin is still open. However, according to Yarmolinsky (1995), the DNA-gyrase is converted into a DNA-damaging agent by the toxin of the TA CcdA/CcdB and could damage the DNA. Also, according to Yarmolinsky, some restriction enzymes and methylase belong to the TA family. So, under stringency, restriction enzymes could cut DNA and cause damage. According to Yarmolinsky, cumulative damages would cause cell death.

The stringent response also induces SOS response (repair of DNA damage) (Nyström 1998). The SOS response is a mutagenesis-inducible response. The induction of the SOS response seems to be proportional to the number of lesions. Damage to DNA may give rise to mutations through the SOS response or lead to cell death (Walker 1984).

A new hypothesis proposes the coupling of these two mechanisms. With damage to (by toxin) and repair of (by SOS response) DNA, the objective of the TA–SOS mechanism could be the production of stochastic mutations and cells that are better adapted to the new conditions. The cost of this mechanism is the death of many cells. Accordingly, a high number of mutations in cell cultures and mutants growing on dying cells are often observed at the onset of the stringent response (Huisman et al. 1996; Zambrano et al. 1993). These mutants seem to be well adapted for survival over long periods of starvation (Huis-

man et al. 1996). Varying environmental conditions favour the rise of mutants showing an increase of fitness (Huisman et al. 1996; Hill and Gray 1988).

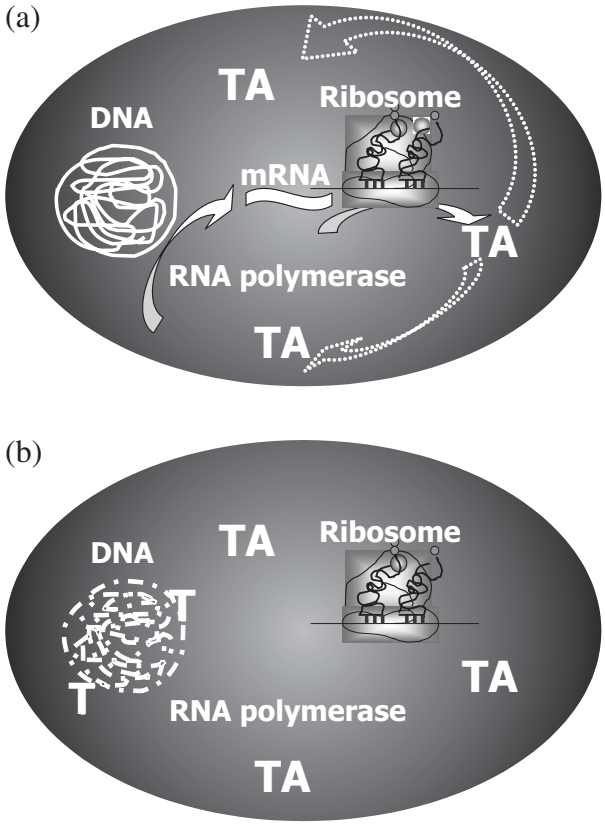
Moreover, cells with DNA injured by toxin could maintain their enzymatic activity but could be incapable of cell division. These cells could be VBNC. Under favourable conditions, DNA repair could regenerate the culturability of these cells.

Also, at the onset of stringent response, the cell downregulates synthesis of RNA, peptidoglycan, lipids, and proteins, and induces proteolysis and synthesis of the proteins that are required for stress adaptation and long-time survival (Nyström 1998). With the stringent response, the decrease of the specific activity and the toxin theory could explain the four states of the cell's viability (viable, dormant, VBNC, dead) that are observed in the literature (Masson et al. 1986).

These observations could reconcile the death school and the dormancy school. At the onset of starvation, the toxin becomes active and causes mutations and cell death. With the onset of the stringent response network, the cells adapt by synthesizing survival proteins and by mutating. When the concentration of toxin has decreased, the adapted cells might survive long periods of starvation. Further studies are required to validate this hypothesis.

In the proposed model, the production of the TA couple is included (Fig. 4). In Fig. 4*a*, the cell is growing and produces TA. Under these conditions, the antitoxin is present and inhibits the toxin. In Fig. 4*b*, the cell is in an endogenous phase. The concentration of antitoxin deceases, and the toxin exerts its bactericidal effect. The fit of the model to the data should validate the hypothesis. However, as mutation is a stochastic process, it would be difficult to model the appearance of cells that are better adapted to the new conditions.

Fig. 4. Regulation of toxin actions. (*a*) Production of the toxin–antitoxin couple during growth; (*b*) action of toxin during starvation. DNA, deoxyribonucleic acid; RNA, ribonucleic acid; mRNA, messenger RNA; T, toxin; TA, toxin–antitoxin.



5.2. Maintenance energy

Survival over long starvation periods implies that under these circumstances, energy expenditures for maintenance become almost negligible. McGrew and Mallette (1962) reviewed the opinions of microbiologists pertaining to cell energy needs. They defined maintenance energy as the energy required to compensate for the wear and tear caused by the action of physical and chemical agents in the cell, i.e., to maintain the status quo.

According to several authors (Pearson et al. 1991; Verduyn et al. 1991; Chudoba et al. 1992) the most significant specific energy expenditure (moles of ATP per 10^{-4} g of cells) is related to protein synthesis. Their data show that close to 70% of the energy expended for macromolecule replacement would be for protein synthesis. According to this theory, maintenance energy would increase with the protein level in the cell.

Kurland et al. (1996) also present a model showing that translation fidelity would depend on the elongation process rate and that the error percentage would increase exponentially above an optimal translation rate. However, Mikkola and Kurland (1991) did not establish a relationship between cellular growth rate and the energy expended to maintain translation fidelity. These authors established a relationship between the maximum growth rate and the rate of the PSS, but they did not observe any relationship with translation errors. Also, during exponential growth, cells seem to maintain good translation fidelity (Nyström 1994).

Based on a model that relies on an ATP balance during various chemostat tests, Pearson et al. (1991) estimated the turnover rate of *E. coli* macromolecules at about 50% of the synthesis rate. Based on these observations, van den Berg (1998) proposes a model describing maintenance using a protein synthesis–decline process (turnover or active breakdown). In this model, the turnover rate of proteins increases with their expression level.

The stringent control network plays a role in maintaining the precision of the genetic transcription; this confirms the idea that metabolic changes occurring during a deficiency mainly target the maintenance of functional proteins (Nyström 1998).

Tempest and Neijssel (1984) consider that maintenance energy is associated largely with the maintenance of the ionic cellular potential. They consider that the renewal of various macromolecular components of the cell is only a small part of the energy required to maintain metabolic activity. According to them, maintaining the driving force of various ions through the membrane could represent a significant part of the maintenance energy. According to Stouthammer and Bettenhaussen (1977), leakages of protons through the membrane and the active trans-

fer would "de-energize" the membrane (without consideration for ATP generation). They also note that proton leakages are less significant and active transfer can be associated mainly with growth activities. We would thus be in a position to believe that the energy spent under aerobic conditions for the renewal of macromolecules would be the main part of the macromolecule maintenance catabolism when there is no uncoupling between anabolism and catabolism.

A diminishing of carbon limitation would generate a loss in efficiency in cellular metabolism (Neissel et al. 1996). The accumulation of certain metabolites would favour the induction of futile cycles (or energy-dissipating processes) and hence the uncoupling between anabolism and catabolism. In fact, several hypotheses were postulated to explain the loss in efficiency of cellular metabolism during an exponential growth phase. Chang et al. (1993) noted an increase in the maintenance/growth ratio when measuring respiration and CO_2 production in exponential growth cultures ($S_0/X_0 > 20$). A few authors (Liu et al. 1999; Liu 1999) propose equations to model the consumption of substrate associated with futile cycles. The Liu (1999) model adequately reflects some values found in the literature, but it does not explain the underlying metabolic processes.

On the other hand, Nilsson et al. (1984) showed that for a given growth rate, the level of mRNA of some constitutive proteins was nine times the level observed for a growth rate that was five times lower. So, as the level of mRNA changes with growth rate, the maintenance of this component could change accordingly. This component should have an impact on maintenance because it has a high turnover.

In short, we can say that under stringent control maintenance is proportional to the mRNA and protein level of the cell. After release of the stringent control, futile cycles would significantly increase maintenance. In a first attempt, the proposed model could include a maintenance term proportional to the protein level in the cell. Futile cycles would be included in the model after further studies.

6. Extracellular factors

Some external factors can modify the evaluation of the actual growth rate of the active cells. They can include the population evolution in mixed cultures and grazing by protozoa or higher life forms, e.g., nematodes.

6.1. Population changes

It is a well-known fact that selection phenomena occur in a continuous culture system at both the species and the mutation levels (Dykhuizen and Hartl 1983). So, in mixed culture systems, the variation of loading is generally accompanied by changes in population structure (Cassell et al. 1966; Grady et al. 1996). Obviously, modifications of kinetic parameters of the biomass are associated with these population changes (Grady et al. 1996; Kovárová-Kovar and Egli 1998).

According to Kovárová-Kovar and Egli (1998), the metabolic capacity (C) of a mixed population is the product of the sum of the number of organisms ($\sum N_i$) responsible for substrate degradation and their respective induction intensity (I_i). Thus, from a mathematical standpoint, if we are able to model the variation in induction intensity, only one variable ($\sum N_i$) would allow the representation of the variation in population structure. Werker and Hall (2001) used microbial fatty acids as an indicator of the population dynamics in activated sludge. Using the variation of the relative distribution of microbial fatty acids, they deduced a single variable to quantify the biomass activity variation. This shows that choosing a suitable model makes it possible to model the population dynamics with a single variable. If the induction of cells is modeled, one part of the growth rate variation would be associated with the induction variation, and another part would be associated with the variation of the population structure. The part of the growth rate variation caused by the population structure variation would be modeled using a single variable, as proposed by Werker and Hall (2001).

According to Dykhuizen and Hartl (1983), for a population composed of two bacterial species, each having one level of activity, the ratio of the two populations varies according to the following equation:

$$[1] \qquad \ln[X_1(t)/X_2(t)] = \ln[X_1(0)/X_2(0)] + (\mu_{obs1} - \mu_{obs2})\,t$$

So, the evolution of a biocenosis, i.e., a biomass composed of several bacterial species, would depend on the difference of the observed growth rates, and its composition would depend on the initial composition of the various species. In fact, the normalized sum of the differences of the observed growth rates could constitute a single variable representing the biocenosis evolution rate. However, the literature review did not reveal data that would permit the deduction of trends. Moreover, according to eq. [1], a single cell might be able to become dominant, given enough time. A single cell has little influence on the overall rate in an activated sludge. After some time, single cell could multiply and her daughters, which would grow more numerous, could have a significant influence on the overall rate. Thus, long-term prediction of the biocenosis and rate of evolution will need continuous data acquisition to take into account the unquantifiable components as a single cell or small differences in the rates that would eventually dominate.

Clearly, further studies are required to find a mathematical formulation for a single variable that reflects or mimics the evolution of the maximal growth rate of a mixed population.

6.2. Predation

In activated sludge systems, protozoan growth may contribute to a significant part of the biomass decay rate (van Loosdrecht and Henze 1999). Protozoans and metazoans may constitute 10% to 20% (w/w) of the biomass (Madoni 1994) and exercise a significant part of respiration (Griffiths 1997). These protozoans and metazoans can directly consume the substrate, consume dead or live bacteria or, as the case may be, other protozoans (Gerardi et al. 1990; Atlas and Bartha 1998).

A bacterial population would develop in a completely different manner if protozoans were not present. From an ecological standpoint, the selective pressure exerted by protozoans modifies the population structure. It seems that the predation of protozoans on free or suspended bacteria favours the growth of flocculent species or, at least, the flocculation of species present (Ratsak et al. 1996; Pike and Curds 1971).

Predation induces cyclic variations in population density (Atlas and Bartha 1998). Protozoan predation is often defined by a Lotka–Voltera type relationship, but experiments rarely support this model (Atlas and Bartha 1998). So, one can ask how to model predation on a flocculated biomass composed of various bacteria, protozoan, and metazoan species that are found in activated sludge. Predation and growth of protozoans appear to be neglected aspects in modeled processes. Knowledge of these organisms represents a field of expertise to be developed. The ASMs simply use an overall decay rate including cell death and predation (van Loosdrecht and Henze 1999). Thus, when the time constant of the transient studied is shorter than the time constant of the variation induced by the predation process, a constant predation rate can be used.

7. Conclusions

Current models assume that cells or biomass have only a single level of expression of specific activity. The experimentally determined parameters are therefore inherently dependent on operating conditions and the configuration of the system studied (Henze et al. 2000). Actually, engineers use standard design rules and their knowledge to choose the process configuration or to optimize the operation of a particular plant. Models are more often used, but in such cases, ASMs are only of some assistance because they do not take into account the metabolic adjustment of the active biomass and the subsequent variation of the parameters. Also, nucleic acid probes are now used more and more often in studies of wastewater treatment processes and microbial ecology (Amann and Ludwig 2000; Wilderer et al. 2002). So, quantification of active cells with probes will require models with further refinement in the description of active biomass because the variation of specific activity would then be taken into account. Thus, a new model would help in understanding the transient behaviour of the activated sludge process and in the design and the operational optimization of the process. With such a model, it should be possible to find the intrinsic value of parameters defining the growth rate of the biomass on a given substrate.

Information from the literature review has been used to create a conceptual model, which is presented in Figs. 1 and 2. The model includes the main metabolic pathways as substrate transport, formation of endogenous reserves, formation of products, and protein synthesis. The model is a simplified view of cell metabolism, but it can produce good trends and was fitted on data of transient behaviour found in the literature (Lavallée et al. 2005).

Here, the aim of the proposed model is to predict the varying growth rate of the active cells. The cell is separated into different components: endogenous substrate, endogenous reserves as glycogen or PHA, and some proteins or enzymes.

To complete the model, the metabolic state of the active biomass is introduced via a simplified representation of the PSS (see Fig. 2). Modeling of ribosome, RNA polymerase, and mRNA levels within the cell could be a first step of a proposed model. In this representation, mRNA is produced by RNA polymerase and is used by ribosomes to produce proteins or enzymes. These enzymes produce the metabolites used by the cell for growth. As discussed earlier, the state of the PSS will set the rate of macromolecule elongation and thus the growth rate of bacteria. These components will model the limitation of the growth rate by transcription under stringency and by translation under fast growth rate conditions.

This description used by Lavallée et al. (2005) fits quite well with the data related to the transients of fast- and slow-growing biomass.

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List of Symbols

C	metabolic capacity
I	induction intensity
N	number of organisms
S_0	initial substrate concentration
t	time
X	biomass concentration
X_0	initial biomass concentration
X_H	concentration of heterotrophic organisms
μ_H	growth rate of heterotrophic organisms
μ_{obs}	observed growth rate of organism