CYBERNETIC MODELING OF THE METABOLIC ADAPTATION OF ACTIVATED SLUDGE BIOMASS

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Abstract: Some publications are indicating that the evaluation of kinetic parameters of Activated Sludge Models (ASM), is influenced by enzymatic regulation. The objective of this paper is to present an activated sludge model which mimics the enzymatic induction at the transcription level of active biomass within the frame of ASM models. The model has been fit on data found in literature. The proposed model gives a more realistic picture of active biomass and of its specific activity, but further research is required to support the model with experimental data. In the end, the objective is that a single set of values for the model parameters would allow to predict the response of different processes over a much wider range of conditions than currently possible. This will eventually extend the model to be an ever more helpful tool in research and understanding of processes in treatment systems. *Copyright © IFAC 2004*.

Keywords: Cybernetic, model, waste treatment, dynamic behavior, rate, regulation.

1. INTRODUCTION

Most activated sludge models (eg. ASM models, Henze et al., 2000) are based on Monod kinetics. Thus, the underlying hypothesis is that cells or active biomass possess only one metabolic state, or level of specific activity.The parameters are thus intrinsically dependent on the operating conditions and the system configuration and have therefore to be evaluated for each of these. This is called calibration and is a major task in modelling activated sludge systems (Vanrolleghem et al., 1999). But as cells regulate their metabolic state according to environmental conditions (Daigger and Grady, 1982a; Grady et al., 1996), significant modifications of the operating conditions or of the process configuration will induce metabolic changes. These metabolic changes are not taken into account in the ASM models, and thus lead to discrepancies between simulations and real process behaviour. Furthermore, it was also shown that evaluation of kinetic constants could be influenced by enzymatic regulation (Vanrolleghem et al., 1998; Çinar and Grady, 2001; Lavallée et al., 2001).

Actually, engineers use standard design rules and their experience to choose the process configuration or to perform the optimisation of the operation of a particular plant. Models are more and more often used, but in such cases, ASMs could only be of some assistance as they don't take into account the metabolic adjustment of the active biomass and the subsequent variation of the parameters. Therefore, engineers interested in making modifications to a process configuration, or interested in optimising operating conditions, possess few tools to predict the behaviour of the real plant after the modifications. Thus, a new model would help in the understanding of transient behaviour of the activated sludge process, and in the optimised design and operation of the process.

Also, nucleic acid probes are more and more often used in studies of wastewater treatment processes

(Wilderer et al., 2002). So, quantification of active cells with probes will require models with further refinement in the description of active biomass as the variation of the specific activity would be taken into account.

Some authors showed transient behaviours of pure cultures as well as activated sludge (Chiu et al., 1972; Daigger and Grady, 1982a and b). The understanding of these transient behaviours (in these cases the variation of the maximal growth rate) remained not well understood as the r-RNA theory could not explain all of these (Daigger and Grady, 1982b). Also, the start up of metabolic processes after sudden substrate addition has not been described entirely by the usual models describing substrate uptake and storage processes (Vanrolleghem et al, 1998; Daigger and Grady, 1982a). According to biochemistry literature, the mechanisms describing the growth process seem to be well understood, but the dynamics of the whole process is still not well defined (Cangelosi and Brabant, 1997; Muttray et al., 2001). So, a new model would help in the understanding of transient dynamics in microbial cultures.

The objective of this research is to propose some modifications to the ASM by introducing enzymatic induction at the transcription level to model the variation of the specific activity of cells. The goal is to get a more realistic representation of active biomass, and therefore a better evaluation of the kinetic parameters. The aim of the proposed model is to model the varying specific growth rate of the active cells. With the proposed representation, parameter identification would become a procedure independent of sludge age, or independent of process configuration which is not possible with the current ASM. So, optimised design or operation of wastewater treatment plants could be done with the help of a better suited mathematical tool.

2. DESCRIPTION OF THE MODEL

Recent works in biochemistry reveal some mechanisms that could help to explain the observed transients. So, the main feature of the new model is that variables are introduced in the model to take these phenomena into account. Based on an extensive literature review, these new variables will model some key components in the description of growth rate. The description of the heterotrophic biomass includes 23 processes, 28 parameters and 16 components. Similar to ASM, the model is presented under a Peterson matrix form. As parameters are linked to processes with different time constants, it is possible to perform independent identification of subsets of parameters with a suitable experimental procedure.

The representation of the biomass is a structured one. Cells will be separated in different components, i.e. intracellular substrate, endogenous reserves such as glycogen or PHA, and some proteins or enzymes. The enzymes produce the metabolites used by the cell for growth and are those describing the reactions shown in Figure 1.

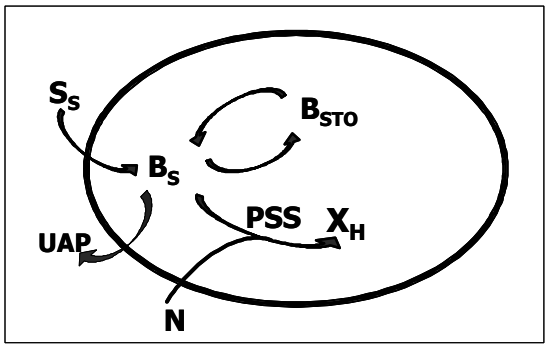

Figure 1 Schematic representation of the main metabolic pathways.

A similar representation was initially proposed by Dircks et al. (2001). In Figure 1, substrate (S_S) is taken up by the cell to form an intracellular substrate (B_S). The cell can grow on this intracellular substrate, make stored products (B_{STO}) or produce utilization associated products (UAP). Nutrients (N) are required for growth of active cells. All of these processes could be induced and regulated at the transcription level as proposed by Ramkrishna and co-workers (Kompala et al., 1986; Turner et al., 1989; Baloo and Ramkrishna, 1991). So, rates associated to each process are dependent of enzyme levels within the cells. However, the induction and repression of the processes is modeled with Michaelis-Menten kinetics as in ASM rather than with cybernetic variables as in Ramkrishna models. So, it is possible to attribute a metabolic meaning to each function. The word cybernetic is used for description of a communication process within the cells.

The new feature of the model is a simplified representation of the protein synthesis system (PSS). The PSS representation is given in Figure 2.

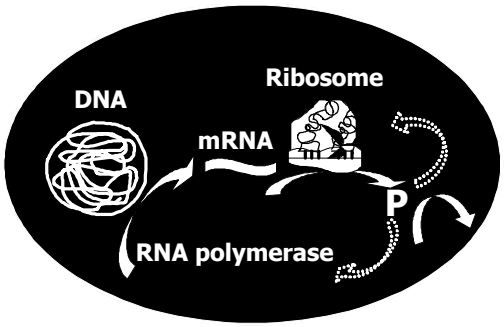

Figure 2 The conceptual model of the PSS.

According to Baloo and Ramkrishna (1991) a component with a short half life is modeled by the variable mR. As messenger RNA (mRNA) has a short half life, it is assumed that the dynamics of mRNA is modeled by this variable. The increase rate of mRNA is regulated by the RNApolymerase level. The concentration of this enzyme will be mimicked by the variable E_G. According to Marr (1991), a component with a longer half life such as stable ribosomal RNA (rRNA), is also modeled by the variable E_G. According to this representation, the protein synthesis is dependent of two main steps. During the first step, the RNApolymerase will perform the transcription of DNA code and build the mRNA. During the second step, ribosomes will translate the message of mRNA and will elongate proteins. For modeling convenience, the initiation of transcription or translation, is all described by a classical Michaelis-Menten kinetic mechanism. The rate of translation is dependent of the mRNA concentration and ribosome subunits (Draper, 1996). Obviously, the real process is much more complex than this representation, and several steps and components are not included here.

So the Monod (1949) equation, largely used in modeling of activated sludge, is modified as follows:

$$\mu_H = \mu_{H\max} \cdot \frac{e_G}{e_G^{\max}} \cdot \frac{mr}{K_{mr} + mr} \cdot \frac{B_S / X_H}{K_{B_S} + B_S / X_H}$$

The ratio B_S/X_H is the concentration of the internal substrate. The ratio e_G/e_G^{max} reflects the specific activity of the active biomass and will change the μ_H value according to the r-RNA level within the cell. The saturation equation including *mr* will change the μ_H value according to the availability of mRNA (Vanrolleghem et al., 1998; Lavallée et al., submitted). Setting the *mr* saturation equation to 1, it is possible to find the Ramkrishna and co-workers equation, and setting also the e_G ratio to 1, one obtains the Monod equation. Thus, the proposed equation is a more general expression of the usual growth rate definition, and is relying on biochemical concepts. Values for each parameter should be chosen in agreement with these meanings and the following time constants. The components mr and e_G will model the limitation of the growth rate by transcription under stringency (i.e. when cells are starved of substrate), and the limitation by translation under fast growth rate (i.e. in excess substrate conditions). Accordingly, after a shift-up, the growth rate will increase quickly with mRNA, and afterwards it will increase slowly with ribosome level, as observed by Kjeldgaard et al. (1958) and Cangelosi and Brabant (1997). This regulation process is shown schematically in Figure 3. Under slow growing conditions, as mRNA has a high turnover, its level will reduce quickly, and according to the Michaelis-Menten kinetic, the ribosomes will become in excess compared to the actual growth rate. This seems in agreement with the observations of Flärdh et al. (1992). So, the schematic representations shown on Figure 2 and Figure 3 are coherent with trends shown in literature. The model is a simplified view of cell metabolism, but can give good trend predictions, and has been fitted on data of transient behaviour found in literature (see below).

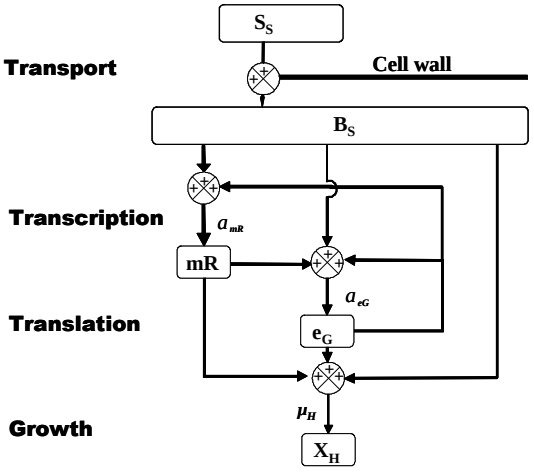

Figure 3 Schematic of the growth rate regulation mechanism.

Some authors made the hypothesis that the ratios of cell wall and membrane per nucleus remain constant for all growth rates (Schaechter et al., 1958; Marr, 1991). Their hypothesis is based on their observations that the ratio "surface areas of cell wall/nucleus" remains virtually constant. So, the same assumption is taken in the proposed model, and the cells will be represented by X_H, the mass of structural components built of one nucleus, and the corresponding fraction of cell wall. As the ratio of these structural components is assumed constant, the evaluation of active cells should be performed by measurement of the DNA concentration. The specific COD of cells will rise and fall with the E_G and *mR* levels. Thus, the variables E_G and *mR* will be used to model the variation of the growth rate and also the rise and fall of biomass COD at the same time.

3. THE PROPOSED EXPERIMENTAL METHOD

Rather than a simultaneous estimation of all parameters at once, the estimation problem is subdivided in different estimations of subsets of parameters. The subsets of parameters are constructed according to their relevant time constant. As *mR* has a short time constant and E_G a larger one, it is possible to perform parameters evaluations successively. Moreover, the fit of process rates and component concentrations (COD/L) has to be done simultaneously. Evaluation of the rates can be performed using oxygen consumption rate (rO_2) measurements, while the evaluation of the state variables is performed using COD measurements. The evaluation of the actual growth rate should be

performed using DNA measurements. The rO_2/DNA ratio should be evaluated to estimate the specific activity or study the variation of the growth rate (μ_H). From this information it is possible to deduce the dynamics of variables such as *mR* (mRNA), and E_G (rRNA). As *mR* has a short half life, a quick variation of μ_H will be caused by *mR*. A slow variation of μ_H will be related to E_G variations as it has a larger half life.

In the experimental results shown below, the biomass was cultivated in a chemostat with a hydraulic residence time of 8 days and glucose was the sole source of carbon. To simulate a start-up, a transient was induced by applying a 5 times dilution of the biomass. The rO2 evaluation was performed with a LSS respirometer (Spanjers et al., 1998). The DNA measurements were performed using bisbenzimide (Paul and Myers, 1982). DNA was extracted according to Muttray et al. (2001). Glucose and glycogen concentrations were evaluated using the anthrone method (Daniels et al., 1994). Growth of protozoa was inhibited by applying an anaerobic period of 3 hours daily.

4. FIT OF THE MODEL

With the chosen representation of active biomass, it is possible to model variations of the growth rate and some other processes (such as storage) within cells. The proposed mathematical formulation is able to describe the variation of the specific activity of active biomass.

In figure 4 it is shown that it is possible to model the transient behaviour of activated sludge during short-term batch experiments (Vanrolleghem et al., 1998).

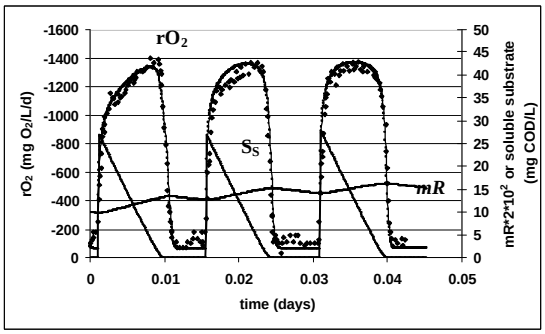

Figure 4 OUR start-up phenomena observed when 3 pulses of S_s are dosed with 22 minutes interval to sludge that was starved for 12 hours (data from Vanrolleghem et al., 1998).

In this experiment, the sludge was first starved for 12 hours. Following the starvation period, 3 pulses of substrate were injected in the respirometer at time 0, 0.015 and 0.030 days. On the first and second pulse one can observe a gradual increase of the rO_2 or, in other words, of the growth rate, until the added substrate is depleted, leading to the sudden drop of rO_2. As *mR* has a short time constant, the model was fitted on these data by changing the increase and decay rate of the variable *mR*. In the experiment depicted in figure 4, the available substrate is used for reconstruction of the *mR* pool after the starvation, raising the specific rO_2. After the second pulse (after 0,025 d), the *mR* concentration has reached the saturation level and the decline of the specific activity associated to a decay of the *mR* concentration is no longer observed in the third pulse data. The proposed analysis is a simplified view of PSS activity, but fitting of the model on this experiment gives good agreement with the data.

In figure 5, several batch experiments that are extending over several hours were fitted with the same set of parameters used in figure 4.

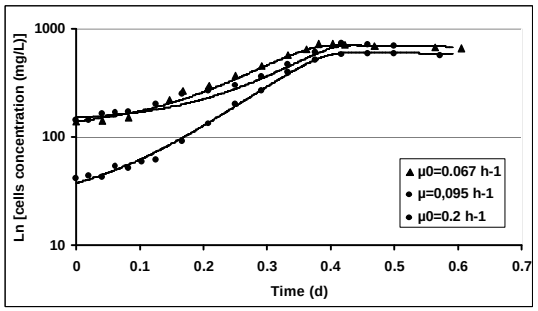

Figure 5 Fit of lag phase (data from Chiu et al., 1973).

In these experiments, mixed cultures with different initial growth rates (μ_0) showed different lag phases (Chiu et al., 1973). Only the initial values of the state variables had to be changed to obtain these fits. The Ks value for substrate uptake was the only parameter that was used as degree of freedom. According to Ferrenci (1999), modelling of two transport enzymes with different Ks values could describe the Ks variation. This feature could be added to the model in the future. The results show that the model is able to predict the transient in short-term experiments as well as in experiments extending over several hours which is not possible with ASM. Consequently, with this model the calibration procedure can be reduced, and several process configurations can be modelled without recalibration of the parameters for each configuration as required with the current ASM.

Modelling of transcription and translation is required to model the growth rate variations in natural populations. These observations are in agreement with Daigger and Grady (1982a) that concluded that physiological adaptation occurred even in the presence of species selection. Of course, other processes as predation, parasitism or selection of particular species are of importance and are

limitations in the use of the proposed model. But the same holds for ASM.

The relative concentrations of E_G, X_H and the growth rate are presented in Figure 6. The cell components are expressed as a ratio to the initial concentration. In this figure one can see that between 0 and 0.15 d, the growth rate increases faster than the E_G component, indicating that the mR variable has an effect on the growth rate. Between 0.15 d and 0.22 d the growth rate increases at the same rate as E_G, indicating that E_G is rate limiting according to the RNA-limiting theory. After 0.22 d the growth rate increases slower than E_G indicating that another process is rate limiting. The exogenous substrate concentration becomes rate limiting ($S_S<2K_S$) only after 0.35 d, and according to the calibration procedure, in such circumstances the substrate uptake is rate restrictive. This analysis is in agreement with the analysis performed by Daigger and Grady (1982b) on the RNA, proteins and DNA increase rates in batch experiments. So, the use of the proposed model could help in the understanding of transient dynamics in activated sludge.

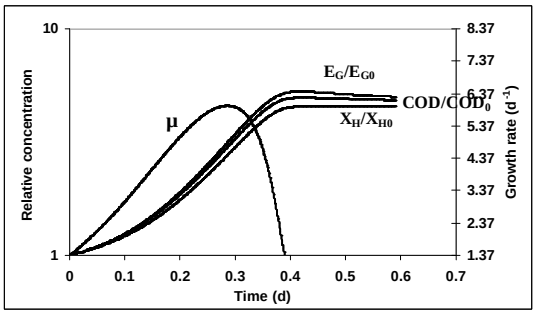

Figure 6 Relative concentrations of components during a batch experiment (μ_0=0,067 h^{-1}).

In figure 7, data from a start-up experiment were fit with the parameters used to perform the fit in the previous figures. Here, the decay of cells and hydrolysis processes were introduced in the model. The response of the model is in good agreement with the COD and glycogen data. However, the increase of active cells concentration (calculated with a COD/DNA ratio) showed a lag time of 8 days, while the fit of the model on these data gives a lag time of only 4 days. Also, the model didn't fit the maximal rO_2 variation (figure 8). So, the model needs some improvement. As the substrate uptake induced respiration, it is expected that the modelling of the successive induction of the dual substrate uptake systems would give a better fit on the specific respiration rate.

So, a single set of parameter values can fit several experiments and simultaneously models the variation of several components of the biomass. Thus, the chosen representation of the cells seems appropriate to model transient phenomena in the activated sludge process. However, further studies are required to define the limitations of this new model, and more experimental data should be collected.

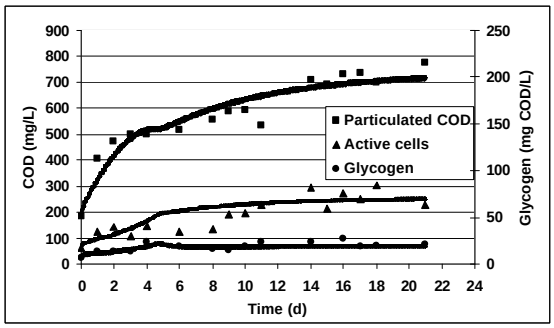

Figure 7 Fit of start-up transient in a chemostat.

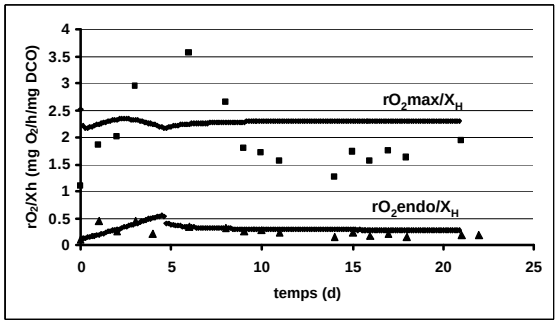

Figure 8 Fit of specific respiration rate on a start-up experiment.

5. CONCLUSION

The proposed model is aimed to increase the understanding of the dynamics or transitory behaviour of wastewater treatment systems. This model will also improve the quality of the kinetic information obtained by parameter estimation. Also, the specific activity description of biomass is expected to make the kinetic constant evaluation a procedure that is independent of sludge age and in many cases, of process configuration. Thus, a single set of values for the kinetic parameters would describe the response of the wastewater treatment processes. This will eventually extend the model to a helpful tool for research, understanding and design of processes in treatment systems.

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