Modelling using rRNA-structured biomass models

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ABSTRACT

Models currently used have been developed to describe the storage response in the activated sludge process. In these models the distribution of the substrate flux between growth and storage is an empirical function. rRNA-structured biomass models are proposed to describe the metabolic status of cells in view of predicting the growth response ($d\mu/dt$) of cells in activated sludge process. The autocatalytic reaction rate of the synthesis of the PSS component (rRNA) can provide a mechanistic explanation for the growth response and the growth lag phase. The proposed models were able to describe and predict properly the growth response of the biomass in various types of reactor. Such models could be more widely applicable by using intrinsic model parameters. This would be a key improvement for as it would lead to improved models for design.

Key words | activated sludge process, biomass, growth response, modelling, rRNA

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INTRODUCTION

Overview of models and structuring in the models

Daigger & Grady (1982*a*) were the first authors to propose a structured approach to model the metabolic status of the biomass in activated sludge processes. They noted that the inputs to most wastewater treatment systems are time-variant, indicating that steady-states are seldom achieved. Consequently, realistic prediction of the performance of biochemical unit processes requires that the dynamic response of the microbial culture be considered. They divided the transient phenomena into two general classes; the storage response and the growth response.

Since then, various models have been developed to describe the storage response in the activated sludge process. The main ones include the IWA Activated Sludge Model No. 2d (ASM2d), ASM3 (Henze *et al.* 2000), ASM3-BioP (Ky *et al.* 2001; Rieger *et al.* 2001) and TUDelft model (TUDP) (van Veldhuizen *et al.* 1999).

For all these models, the values of the kinetic parameters are typically site-specific and calibration is usually required for accurate description of the actual process under study (Gernaey *et al.* 2004). Usually and fortunately, the calibration effort is limited to a few key parameters and some steps in the calibration procedure can be omitted depending on the aims (Gernaey *et al.* 2004).

In ASM, the biomass is separated into micro-organisms and different storage compounds. It is assumed that the

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various intracellular fractions (but storage) of the micro-organisms does not change according to the pseudo steady state hypothesis.

However, when the conditions in the process significantly change, the level of intracellular components as well as the rates and the metabolic status of bacterial cells will change. Hence, one pseudo steady state can not describe the old and the new situations. Then, recalibration of the kinetic parameters involved is usually required for accurate description of the process exposed to the new operational conditions or for accurate description of the new process configuration (van Loosdrecht & Heijnen 2002; Gernaey *et al.* 2004).

The simplifications adopted in conceptual models of the metabolism are dependent on the experimental methods used to determine the parameters value, and therefore the conceptual models are empirical by nature and the parameters value are often site specific. Due to the site-specific nature of the calibration procedure, the parameters have been denoted as *extant* as opposed to universal parameters that would be *intrinsic* (Grady *et al.* 1996). By introducing the *extant/intrinsic* nomenclature, Grady *et al.* speculated that most parameters are extant because the models fail to consider the metabolic status of the bacterial biomass, suggesting that considering the metabolism could allow the development of more widely applicable (intrinsic) model parameters. It appears that the metabolic status of the cell can be partly captured by considering the level of rRNA per cell (Daigger & Grady 1982*a*).

Consequently, RNA-based models have been developed to describe the metabolic status of cells in view of performing simulations of the microbial adaptation and growth response of cells. Using a simplified RNA-based model, van Loosdrecht & Heijnen (2002) showed the overall trends of the model output for various values of specific enzyme (RNA) decay rates and operational conditions in an SBR. The simulation showed that the parameters had only a limited influence, but also that the model correctly predicted the PHB fractions and growth rates under various sludge retention times.

More recently, two quite similar approaches have been suggested to use molecular data for better description of transient behavior. A first approach uses stoichiometric modelling involving a carbon mass balance and RNA-based molecular techniques to model PHB accumulation and growth of biomass in SBR's (Frigon *et al.*, submitted). The second one uses dynamic modelling involving a COD mass balance and DNA molecular techniques to model the transients induced by substrate pulses and batch experiments with high initial substrate to biomass ratio (Lavallée *et al.* in preparation). Both approaches proposed similar structures.

The discussion in this paper aims to expose these works in progress in order to extend the application range of the ASMs and TUDP to different processes configurations or to different steady states (including the modelling of the transient from one steady state to the other) without the need for recalibration. The objective is not to calibrate the models on a particular data set, but to indicate when a RNA-structured biomass model is needed.

SCOPE AND OBJECTIVES OF rRNA-STRUCTURED BIOMASS MODELS

Faced with different problems of the kind presented above, various authors attempted to develop model in which they structured the biomass COD in specific pools and specifically described the COD conversion between these pools. A survey of activated sludge structured-biomass models indicates the following specific objectives for the developed models:

- implementing a fundamental metabolic yield coefficients (the ATP/NADH2 ratio in the oxidative phosphorylation) (Smolders *et al.* 1994; Beun *et al.* 2000; Dicks *et al.* 2001)
- predicting transient conditions under the dynamic conditions of activated sludge processes or batch tests (Daigger & Grady 1982*b*; Smolders *et al.* 1995*a*, 1995*b*; Grady *et al.* 1996; Beun *et al.* 2000; Lavallée *et al.* 2002; Vanrolleghem *et al.* 2004; Frigon *et al.* 2006)
- predicting microbial activities in different processes (Oerther *et al.* 2001; Frigon *et al.* 2002*a*; 2002*b*; Stroot *et al.* 2005; Simpson *et al.* 2006)
- interfacing with microbial population dynamics results obtained by molecular techniques (Frigon *et al.* 2006; Gilbride *et al.* 2006)

RNA-based models have been developed with all these specific objectives in prospect.

S*toichiometric modelling* used to describe yields, and *kinetic modelling* used to describe dynamics, are two basic aspects of modelling. Though not independent, the considerations for both modelling approaches invoke different aspects of microbial metabolism and the contribution and promises of these two approaches will be described separately. rRNA models proposed by the authors include structured descriptions of the metabolism in both approaches.

STOICHIOMETRIC MODELLING

The approach proposed by the group at the Technical University of Delft was to model the EBPR microbial activity by describing the stoichiometry of the specific metabolic pathways involved. This metabolic description has the benefit of adding constraints to the model solution space by linking pathways through the production or consumption of energy (ATP) and reducing equivalent carriers (NADH). The external observed yield coefficients for different cellular fractions are all dependent on one metabolic yield coefficient: the oxygen (or NADH2) to ATP ratio. Consequently, assuming that the carriers do not accumulate in bacterial cells allows the expression of the substrate consumption and intracellular component formation rates with a reduced number of *independent* parameters. This property has made it possible to successfully describe full-scale domestic wastewater treatment plants of numerous configurations by adjusting only 3 or 4 parameters (Oehmen *et al.* 2007).

Note further that, since the pathways are all linked through the energy carriers, the successful calibration of such a model is by itself a validation of the proposed model structure.

KINETIC MODELLING–RNA-BASED APPROACH

The cells' composition in relation to the growth rate has been studied by several authors (Herbert 1958; Herbert 1976; Daigger & Grady 1982*b*; Esener *et al.* 1982; Bremer & Dennis 1996). Herbert (1976) observed that when the carbon source is the limiting substrate, the elementary composition of cells is independent of the growth rate, but the fractions of the cell constituents vary.

Approximately 50% to 65% of the mass of fast growing bacterial cells is accounted for by proteins and 10 to 20% is accounted for by RNA. All RNA can be considered rRNA since it comprises approximately 85% of the cellular RNA at all growth rates (Bremer & Dennis 1996) and all proteins are synthesized by ribosomes. The ribosome is the catalytic unit responsible of protein synthesis and its main catalytic constituent is rRNA. As a result, a major aspect of the metabolic status of bacterial cells is the level of rRNA, and its level is correlated with growth rate (Herbert 1958; Koch 1970; Keener & Nomura 1996).

Determination of the RNA/DNA ratio has been proposed to assess the nutritional condition of larval fish (Kaplan 2001) and growth rates in marine bacteria (Kerkhof & Ward 1993; Dell'Anno *et al.* 1998) or in foodstuffs (Milner *et al.* 2001). Muttray *et al.* (2001) used the rRNA/rDNA ratio to characterize the metabolic activity of *Pseudomonas abietaniphila* in activated sludge.

Using a genetic knockout mutant Frigon *et al.* (2006) observed that the reactor configuration and the ability to produce PHB had an impact on the rRNA level for a pure culture growing in an acetate-fed reactor. Because the intracellular concentration of rRNA varied with process configuration or with the loading as well as with the growth rate, these observations showed that one pseudo steady state can not hold for different process configurations or for different steady states (i.e. different maximal growth rates) when a mass balance around rRNA is not included, and then recalibration of the model is required.

RNA-based models have been developed to describe the metabolic status of cells with a view to perform simulation of microbial adaptation and transient behavior of cells (Turner & Ramkrishna 1988; Frigon *et al.* 2002*b*; Frigon *et al.* submitted; van Loosdrecht & Heijnen 2002; Gupta *et al.* 2005; Lavallée *et al.* 2005; Lavallée *et al.* in preparation). In these models, the size of the component, here called the protein synthesis system (PSS, or rRNA), is used to describe the metabolic status of the cells. This component (X_{PSS}) denotes the rRNA level in the cells and it is used to model the modulation of the specific growth rate. It catalyzes the cell constituent synthesis.

Most of these models suggest that the synthesis rate of rRNA is autocatalytic as its rate of synthesis is related to its own concentration (Figure 1). This provides a sigmoidal increase of rRNA level after a switch in conditions. As a result, when the level of the rRNA is low, the rate of change of the transient response is slow; when the level is medium the rate of change of the transient becomes fast; and when the rRNA level is high, its further increase is small and the response doesn't quite change. Accordingly, Daigger & Grady (1982*b*) observed that the rate of change of the transient response ($d\mu/dt$) of *Pseudomonas putida* increased with the growth rate to reach a maximal value near a growth rate of $0.2\,h^{-1}$, and then decreased as the growth rate increased. The autocatalytic reaction rate of the rRNA synthesis can provide a mechanistic explanation for the pattern of the growth rate adaptation observed by Daigger & Grady (1982*b*) and so for the lag phase occurring in a slow growing culture when the substrate limitation is removed.

All proposed models present a component (X_B) to mimic the level of the building blocks or precursors. The synthesis of the PSS and the structural component of the biomass (X_C) (mainly proteins, lipids and DNA) is rate-dependent on the building block level.

Interfacing the biomass composition with molecular techniques

van Loosdrecht & Heijnen (2002) first proposed a simplified model in an academic context. These authors explained that cells prioritize growth to storage. In order to describe the storage process, it is needed to define models with a variable amount of anabolic enzymes. They showed that the simplified model predicted fairly well the PHB fraction in biomass for various SRT.

Frigon *et al.* (submitted) proposed to use a carbon mass balance, PHB extraction, protein assay and RNA molecular techniques to model PHB accumulation and growth of biomass in SBR. These authors proposed a detailed description of the PSS and the structural components of biomass to provide an explicit link between molecular techniques and the model predictions.

Lavallée *et al.* (in preparation) on the other hand used a COD mass balance, glycogen and DNA extraction to model the response to substrate pulses and batch experiments with high initial substrate to biomass ratio. Lavallée *et al.* (in preparation) modelled the PSS using a component with zero mass to conserve a closed COD mass balance on the different components. These authors chose DNA for quantitative population assessment since the DNA level per cell remains constant for all growth rates and can be used for quantitative interpretation of data for bacterial populations in activated sludge.

Table 1 summarizes the components considered by these authors for their RNA-structured biomass.

The two last models were developed with an experimental perspective in view of developing activated sludge models that can be calibrated with *intrinsic* model parameters.

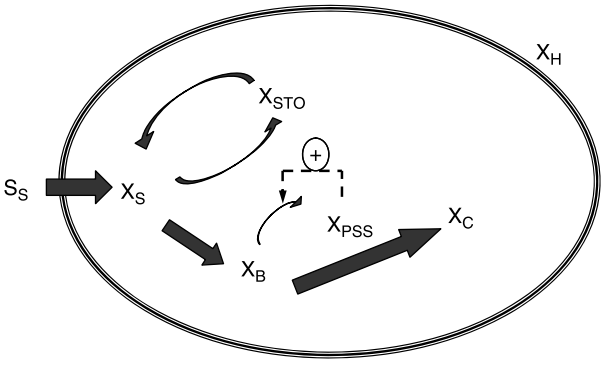

Figure 1 | Common kinetic scheme of the models (symbols used may differ for the different models in literature). S_S: Soluble substrate; X_S: intracellular substrate or metabolites; X_B: Building blocs or precursor; X_{PSS}: protein synthesis system or rRNA; X_C: Structural component of cell or proteins; X_H: $X_S + X_{STO} + X_B + X_{PSS} + X_C$.

COMMON STRUCTURES IN PROPOSED MODELS

A simplified rRNA-structured model was formulated to evaluate the sensitivity of the output of a rRNA-structured model to the parameters of the rRNA synthesis and decay models. Not all usual metabolic processes are included in the model here, since the aim was to explicitly determine the sensitivity of the proposed model structure.

The production of the PSS is an autocatalytic process since it depends on its own concentration. The production rate of the PSS is given by the net synthesis rate minus the decay rate of the PSS and minus the loss causes by cell decay.

Table 1 | Description of the RNA-structured biomass

	Frigon *et al.*	Lavallée *et al.*	Van Loosdrecht & Heijnen
Mass balance	Carbon	COD	COD
Substrate	Acetate	Glucose	Acetate
X_S	acetyl-CoA	G6P	None
X_B	amino acids, nucleic acids, lipids, other precursors	amino acids, other precursors, soluble proteins, etc.	None
X_{STO}	PHB	Glycogen	PHB
X_{PSS}	$X_{PSS,R}$ = RNA $X_{PSS,P}$ = r-proteins $X_{PSS} = X_{PSS,R} + X_{PSS,P}$	X_{PSS} = component with zero mass‘	X_{PSS} = component with zero mass
	(r_{PSS} = autocatalytic)	(r_{PSS} = autocatalytic)	(r_{PSS} = biomass dependent)
X_C	$X_{C,L}$ = lipids, non-proteins and non-RNA cell constituents X_P = proteins $X_C = X_{C,P} + X_{C,L}$ $X_{C,P} = (1 - \gamma) \times X_P$ $X_{C,L} = \beta \times X_P$	X_C = proteins, lipids, cell wall, etc. $X_C = \frac{DNA}{f_{DNA}}$	X_C = proteins, lipids, cell wall, etc. $\Delta X_C = \frac{Y_{SP}}{Y_{SX}} \times \Delta PHB$

$$r_{PSS} = k_{PSS} \times X_{PSS} \times M_i \times M_{i+j} - b_{PSS} \times X_{PSS} - b_{XC} \times X_{PSS} \quad (1)$$

where b_{PSS} and b_{XC} are the decay rate of X_{PSS} and X_C respectively, and M_i are saturation kinetic terms (e.g. Monod).

The X_{PSS} production will be in equilibrium with its decay and its dilution into new biomass. The balance equation for this component into the cell is described by:

$$\frac{df_{PSS}}{dt} = 0 = k_{PSS} \times f_{PSS} \times M_{X_B} \times M_O - b_{PSS} \times f_{PSS} - f_{PSS} \times \frac{dX_C}{X_C \times dt} \quad (2)$$

In this equation, the right term represents reduction of f_{PSS} caused by dilution from growth of the cell population. At steady state, if we assumed the oxygen and the building blocks are not limiting, the equation can be rewritten:

$$k_{PSS} \times f_{PSS} - b_{PSS} \times f_{PSS} = \mu_{max}^{int} \times f_{PSS} \quad (3)$$

In this equation, μ_C^{int} is the specific production rate of the PSS.

The metabolic status of the cells is introduced in the model with the PSS level to describe the growth rate of the cell's structural material (X_C):

$$\mu_C = \mu_C^{int} \times \frac{f_{PSS}}{f_{PSS}^{max}} = (k_{PSS} - b_{PSS}) \times \frac{f_{PSS}}{f_{PSS}^{max}} \quad (4)$$

As shown in Figure 2, using the autocatalytic process for the X_{PSS} production, it was possible to roughly describe the rate of change of the transient response ($d\mu/dt$) of *Pseudomonas putida* observed by Daigger & Grady (1982*b*). The decay rate was varied unless f_{PSS} goes to zero with the dilution rate. From a metabolic point of view, several phenomena can have incidence on the f_{PSS} fraction at low dilution rate. Some authors report variable decay rate, variable maintenance, induction and repression of metabolic pathways etc (Lavallée *et al.* 2002; Lavallée *et al.* 2005).

A complete mathematical description of the simplified model is given under a Petersen matrix form in appendix. In brief, the exogenous substrate is taken up by biomass to yield intracellular substrate (X_S). The intracellular substrate is used for building block (X_B) production, for storage (X_{STO}) and for energy expenditures (OUR)

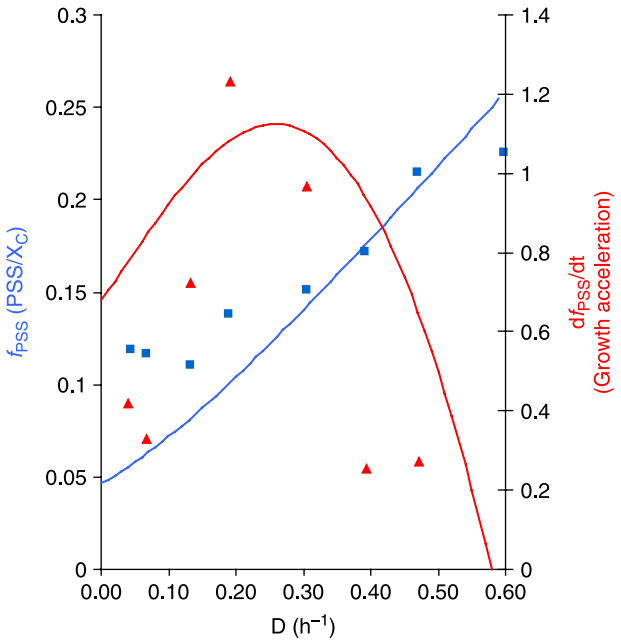

Figure 2 | Variation of RNA fraction and growth acceleration. D = μ-b. The decay rate was varied between 0.02 h^{-1} at D = 0.6 to 0.13 h^{-1} at D = 0.00. (Data adapted from Daigger & Grady 1982*b*).

associated with biomass growth. It decreases with the endogenous respiration of biomass b_{XC}. The PSS (X_{PSS}) is a unit less component.

Figure 3 shows that the model response is sensitive to the initial f_{PSS} value. The output of the model is insensitive to f_{PSS} during the first few hours, but the discrepancy of the cell concentration (X_C) between the different simulations increased all along the batch simulation according to the f_{PSS} used.

The lower the initial f_{PSS} was, the longer the lag phase. Since the rRNA level is correlated to the growth rate at steady-state, a culture growing more slowly will have a lower f_{PSS}/f_{PSS}^{max} ratio and the lag phase will therefore stretch over a longer period, as predicted by the model.

Figure 4 shows the output of the model after an up-shift of the flow rate into a reactor. The cells concentration (X_C) and the f_{PSS}/f_{PSS}^{max} reached the same values for every value of f_{PSS}^{max}. The simulation shows that after 12 days, the f_{PSS}/f_{PSS}^{max} ratio always reaches the same value and the model is thus insensitive to f_{PSS}^{max} at steady state. Again, during the transient induced by the flow rate up-shift, the model was sensitive to the initial f_{PSS}/f_{PSS}^{max} fraction. This indicates that the growth response of the model varies

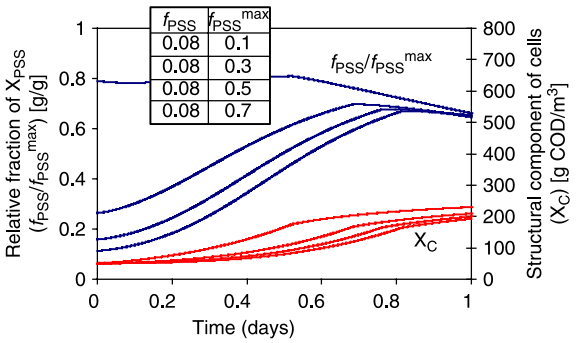

Figure 3 | Batch reactor. S_0/X_0 = 10. Sensitivity analysis for initial values of f_{PSS}/f_{PSS}^{max} = 0.8; 0.27; 0.16; 0.11 units/g.

with the f_{PSS} fraction, that is to say, with the history of the cell components.

Additionally, a phase analysis shows that at steady state, the X_{PSS} level increases when the hydraulic retention time is decreased (Figure 5). The growth response will change accordingly, as well as the transient behaviour after a modification of the retention time. Hence, a rRNA model would reflect the specific growth rate level of micro-organisms, according to the conditions prevailing in the reactor.

In ASMs, the specific heterotrophic oxygen uptake rate can be described by the following equation:

$$\frac{OUR}{X_H} = \mu_H \frac{(1 - Y_H)}{Y_H} \quad (5)$$

In this equation, μ_H and Y_H are two parameters. Because μ_H does not reflect the variations of the specific activity associated to variations of the rRNA level, recalibration of the model will be required when the conditions

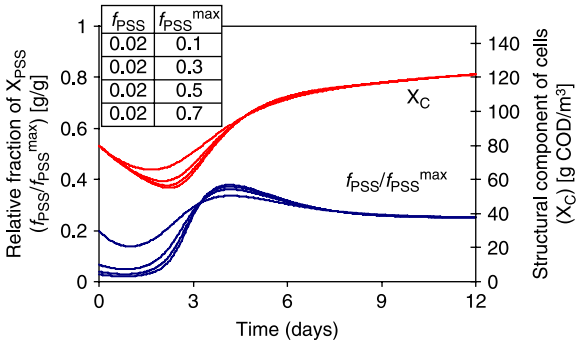

Figure 4 | Sensitivity of the model during the start-up of a continuous-flow stirred reactor (HRT = 6.5 days).

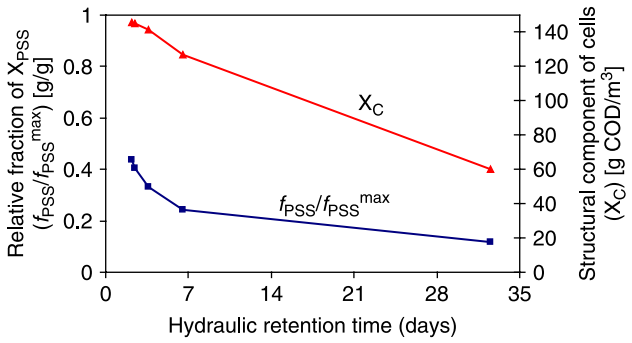

Figure 5 | Semi-continuous-flow stirred reactor. Sensitivity of the model to hydraulic retention time ($f_{PSS}^{max} = 0.5$ units/g).

prevailing in the reactor are significantly different from those prevailing during the preceding calibration exercise.

In a rRNA model, on the other hand, the specific heterotrophic oxygen uptake rate related to the growth process is given by:

$$\frac{OUR_G}{X_C} = \frac{\mu_C^{max}}{f_{PSS}^{max}} \times \frac{X_{PSS}}{X_C} \times \frac{(1 - Y_C)}{Y_C} \quad (6)$$

The parameter Y_C is the yield of biomass on intracellular substrate X_S. This equation reflects the variation of the specific activity of microorganisms and thus recalibration will no longer be required. The calibration procedure would remain similar to those used with ASM, but the reference unit used in the description of the specific respiration would be the PSS:

$$\frac{OUR_G}{X_{PSS}} = \frac{\mu_C^{max}}{f_{PSS}^{max}} \times \frac{(1 - Y_C)}{Y_C} \quad (7)$$

In light of this analysis, it can be concluded that a rRNA-structured model is sensitive to the history of the X_{PSS} component, to the process configuration and to the operation conditions. However, the model is not sensitive to the f_{PSS} fraction at steady state. Thus, identification of the f_{PSS}^{max} value must be done under transient conditions. Since the model is sensitive to the relative fraction f_{PSS}/f_{PSS}^{max}, an explicit link between measured and predicted rRNA level can been made.

MASS BALANCE DURING TRANSIENT PERIOD

Lavallée *et al.* (in preparation) used DNA as basic unit for biomass quantification. The maximal growth rate of cells (μ_C^{max}) was then determined by monitoring the evolution of the DNA concentration in a batch experiment with a high initial S_0/X_0 ratio. The DNA measurements were converted to COD by using a measured DNA/COD ratio (f_{DNA}). Hence, using the COD of the suspended solids minus the COD of glycogen, these authors were able to observe variations of the intracellular COD fractions ($f_{XS} + f_{XB}$). Considering the nitrogen assimilation and the N/COD ratio (i_{nxc}), they observed that f_{XS} was negligible and most of the intracellular fraction was under the f_{XB} form. Using this method for a batch experiment, a significant increase of the f_{XB} fraction was observed as soon as the storage capacity of the biomass was fully utilized, and its decrease after the substrate was exhausted. Since a COD mass balance was used, the nature of the f_{XB} could not be determined. It was assumed that the product was an aminated storage component (e.g. amino acids, intracellular soluble proteins, etc). After exhaustion of the substrate, growth occurred on the basis of a breakdown of glycogen and X_B. With this in mind, Lavallée *et al.* suggested that a model assuming a constant cell size ($X_S + X_B + X_C = Cte$) can be used as long as the storage capacity of the biomass is not fully used. These authors proposed to use a mass balance on the X_B component to model large transients.

Frigon *et al.* (submitted) used a carbon mass balance and proposed to describe the X_{PSS} into two subset pools, the $X_{PSS,R(RNA)}$ and the $X_{PSS,P}$ (protein) in order to explicitly link measured and predicted rRNA levels. The ratio between these two components was assumed constant since these components are tightly co-regulated by cells (Nomura 1999).

Hence, by combining the methods proposed by the authors of these two papers (adapted to the units used) it will be straightforward to determine the f_{PSS}^{max} by using the equation (7), knowing the actual rRNA concentration, the OUR_G and the yield Y_C. Intracellular levels of the component X_B can be determined by using a mass balance of carbon (or COD) and nitrogen around biomass. It follows that a calibration of the model conversion rates is possible on the basis of the above mentioned data.

BIODIVERSITY AND RNA-STRUCTURED BIOMASS MODEL

Proper interpretation of the rRNA level in terms of the cell's metabolic status clearly needs further research. For example, Frigon *et al.* (2002*a*) determined the diurnal variations of rRNA levels of a population of *Acinetobacter* and of a population of *Gordonia* in the mixed liquor of a full-scale wastewater treatment plant, and observed two different profiles: the variations for the first population followed variations in the influent loading, while the rRNA of the second population remained stable throughout the day. Using a model-based analysis, they suggested that the ecological function of the populations may be responsible for the observed trends. Muttray *et al.* (2001) suggested it cannot be assumed that the positive linear relationship between growth rate and RNA:DNA ratio holds for all microorganisms, and also observed that a direct deduction of actual growth rates in batch cultures from the RNA:DNA ratio measured at steady-state may not always be possible.

Additionally, it is increasingly recognized that advances in wastewater treatment processes will require description of the biodiversity of microorganisms present in these systems, and of their diverse biochemical and metabolic activities. With the advent of ribosomal RNA (rRNA)-based molecular techniques, it has become possible for environmental engineers and scientists to accurately assess the bacterial diversity in activated-sludge systems (Yuan & Blackall 2002; Saikaly *et al.* 2005). No wonder that the last decade one saw the publication of several studies describing bacterial diversity in activated sludge process through phylogenetic analysis (evolutionary-based analysis of marker genes), mainly of the 16S rRNA gene. Using rRNA terminal restriction fragment analysis, some indices were proposed to model the fingerprint of a bacterial community structure, that were correlated to influent variables and performance indicators (Gilbride *et al.* 2006).

CONCLUSIONS AND PERSPECTIVES

Structured biomass models are by nature less empirical than unstructured biomass models. While increasing model complexity, they simplify the model calibration effort because they replace extant by intrinsic parameters. Development of molecular techniques has created a deeper insight in the microbial diversity and activity level of microbial populations in activated sludge and, with this insight, RNA-based models are currently under development.

An explicit link between molecular techniques and the model structures have been proposed. It has become possible to determine the rRNA to biomass ratio to accurately assess the metabolic status of microbial populations in activated-sludge systems. The autocatalytic process proposed to model the rRNA component dynamics can describe the rRNA evolution, the growth rate adaptation ($d\mu/dt$) and the growth lag phase observed by Daigger & Grady (1982*a*,*b*) and observed by the authors in their own experiments (Frigon *et al.* submitted; Lavallée *et al.* in preparation). Further studies are needed to provide a proper interpretation of rRNA levels in terms of metabolic status.

A simple rRNA-structured model has been presented here. By combining the methods proposed by the authors, and knowing the actual rRNA concentration, the OUR_G and the yield Y_C, it will be straightforward to determine the by using the equation (7). To model large transients, the intracellular concentrations of the component X_B need to be determined by mass balancing the carbon (or COD) around the biomass. A calibration of the remaining model parameters can then be done by using available data. However, the regulation of the transformation reaction rates of the components are not yet well defined, and it has great influence on the model output. Further studies are therefore need for validation of such a model.

Results from this kind of metabolic modelling exercises and from the TUDP EBPR metabolic model suggest that it may be possible to develop activated sludge models calibrated by *intrinsic* and not *extant* model parameters.

For the time being, however, the rRNA-structured biomass models remain restricted to research. Though, the driving force for that kind of model currently remains low in treatment of wastewater, they present the potential of key improvements that practitioners would benefit from as they would lead to improved reliability of activated sludge models. Water professionals that develop strategies to control microbial populations would clearly take advantage of such models too.

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APPENDIX

Rates	X_{PSS}	S_O	S_S	X_S	X_B	X_{STO}	S_{nh}	X_C	X_{ii}
Units:	units/g	gCOD/m³	GCOD/m³	gCOD/m³	gCOD/m³	gCOD/m³	gN/m³	gCOD/m³	gCOD/m³
r1		$-(1-Y_S)$	-1	Y_S					
r2				-1		1			
r3				-1	1		$-i_{nxc}$		
r4		$-(1-Y_C)/Y_C$		$-(1-Y_C)/Y_C$	-1			1	
r5	1								
r6				1		-1			
r7				1	-1		i_{nxc}		
r8	-1								
r9	$-X_{PSS}/X_C$	$-(1-fu)\times(1+X_S/X_C+X_{STO}/X_C+X_B/X_C)$		$-X_S/X_C$	$-X_B/X_C$	$-X_{STO}/X_C$	$(1-fu)\times(X_B/X_C+1)\times i_{nxc}$	-1	$fu\times(1+X_S/X_C+X_{STO}/X_C+X_B/X_C)$

Rates			
r1	Substrate uptake	$k_S \times Mss \times Mso \times Ixs \times X_C$	gCOD/m³/d
r2	Substrate storage	$k_{STO} \times Mssto \times Msto^{max} \times X_C$	gCOD/m³/d
r3	increase of X_B	$k_B \times Mnh \times Mxs \times Mso \times X_C$	gCOD/m³/d
r4	aerobic growth of X_C	$\mu_C^{int}/f_{PSS}^{max} \times X_{PSS}/X_C \times Mso \times Mxs \times Mxb \times X_C$	gCOD/m³/d
r5	aerobic growth of X_{PSS}	$k_{PSS} \times X_{PSS} \times Mso \times Mxs \times Mxb$	units/m³/d
r6	degradation of Xsto	$Dksto \times Msto \times X_C$	gCOD/m³/d
r7	degradation of Xp	$b_{Xp} \times X_P$	gCOD/m³/d
r8	decay of X_{PSS}	$b_{XPSS} \times X_{PSS}$	units/m³/d
r9	decay of X_C	$b_C \times X_C$	gCOD/m³/d

Mss	$S_S/(S_S + Kss)$
Mso	$S_O/(S_O + Ko)$
Ixs	$Ki_{xs}/(Ki_{xs} + X_S/X_C)$
Mnh	$Snh/(Knh + Snh)$
Mxs	$(X_S/X_C)/(Kxs + X_S/X_C)$
Mssto	$(X_S/X_C)/(Kxs,sto + X_S/X_C)$
$Msto^{max}$	$(f_{sto}^{max} - X_{STO}/X_C)/(Kisto + f_{sto}^{max} - X_{STO}/X_C)$
Msto	$(X_{STO}/X_C)/(Ksto + X_{STO}/X_C)$