Oligonucleotide probe hybridization and modeling results suggest that populations consuming readily degradable substrate have high cellular RNA levels

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Abstract Analyses based on ribosomal RNA (rRNA)-targeted hybridization performed in our laboratory identified two types of bacterial populations: a population with a high RNA level per biomass and a population with a low level of RNA per biomass. To extend these descriptions, the diurnal dynamics of the RNA pool were monitored by rRNA-targeted oligonucleotide probe membrane hybridization. Under the typical diurnal variation in COD loading rate experienced by municipal wastewater treatment plants, the RNA level of the bacterial population with a high level of RNA per biomass varied with changes in the COD loading rate. Under the same conditions, the RNA level of the population with low RNA level per biomass remained constant. A structured biomass model was developed to describe these data. Substrate COD was divided into a readily biodegradable and a slowly biodegradable COD fraction. It was assumed that two specialized populations coexist in municipal activated sludge treatment systems. One population consumes readily degradable COD and the other consumes slowly degradable COD. According to model simulations, the population consuming readily degradable COD has a high level of RNA per biomass under variable substrate concentrations. Comparatively, the population consuming slowly degradable COD has a low level of RNA level per biomass. Furthermore, model simulations reproduced the two diurnal RNA profiles observed in a full-scale municipal activated sludge system. Therefore, we suggest that two populations can be distinguished in municipal activated sludge systems: a population consuming readily degradable substrate and a population consuming slowly degradable substrate.

Keywords Activated sludge; microbial ecology of activated sludge; municipal wastewater; oligonucleotide probe hybridization; rRNA; stuctured biomass model

Nomenclature

Variables describing COD pools

S Soluble or readily degradable COD (g m^{-3})

X Particulate or slowly degradable COD (g m^{-3})

Subscripts describing COD pools

A Active biomass (sum of C and PSS pools)
B Cellular building blocks
C non-PSS cell components
H Substrate from hydrolysis
I Inert COD
L Lipids and biomass components other than RNA and proteins
O Oxygen
P Proteins
PSS Protein synthesis system
R RNA
S Substrate from influent
SS Populations consuming readily degradable substrate (RDS, S_S)
XS Populations consuming slowly degradable substrate (SDS, X_S)

Variables describing reaction rates

b Endogenous respiration constant (d^{-1})
f Fraction of biomass that is non degradable
Y Yield (g g^{-1})
α Ratio among PSS cell components

K Half-saturation constants (units depend on the process)
k rate constants (g g^{-1} d^{-1})
β Ratio among non-PSS cell components

Subscripts describing reaction rates

G Growth, production of new biomass
H Hydrolysis
U Uptake

Introduction

Two types of ribosomal RNA (rRNA) targeted hybridization techniques have been used to determine the identity and abundance of microbial populations in activated sludge systems: fluorescence *in situ* hybridization (FISH) and membrane hybridization. While the data obtained with these two techniques often are expressed as relative abundances, the units of the values used to calculate these relative abundances must be considered in interpreting the results (Oerther *et al.*, 1999). FISH data are expressed as ratios of population cell numbers per total cell number in the sample. These data can be converted to ratios of biomass per mass of mixed liquor suspended solids (MLSS) assuming the relationship between cell abundance and biomass level has been determined for the population of interest. Biomass-based units can be used directly for calibrating and verifying activated sludge models (Oerther *et al.*, 1999). Membrane hybridization data are expressed as ratios of the mass of rRNA associated with a population per total MLSS rRNA. Because the level of rRNA is proportional to the growth rate of a bacterial cell (Herbert, 1961; Veldkamp, 1976), membrane hybridization results are influenced by both the number of cells and the growth rate of a population. Typically, this result is interpreted as the metabolic contribution of the population to the community.

FISH and membrane hybridization techniques are frequently used side-by-side in our laboratory to understand the dynamics of microbial communities in activated sludge systems. Studying different activated sludge systems, two types of population were observed by comparing the percentages determined by FISH and membrane hybridization. In one system, the proportion of *Gordonia* spp. biomass determined by FISH was high (up to 20% of biomass), but the proportion of rRNA determined by membrane hybridization was low (up to 1.4%; (Oerther *et al.*, 2001) suggesting a low level of rRNA per biomass. In another system, the relative cell abundance of *Acinetobacter* spp. was low (up to 4.4%), but the proportion of rRNA was high (up to 43%) suggesting a high level of rRNA per biomass (Oerther *et al.*, in prep.). Since the cellular rRNA level appears to be approximately constant for bacteria growing at the same rate and at the same temperature (Herbert, 1961; Veldkamp, 1976), these results suggest that the dynamics of the rRNA pool are different for each population present in activated sludge. However, the mechanisms underlying these differences are unclear. This paper presents the possibility that the reasons for these observations involve the specialization of bacterial populations in consuming either readily or slowly degradable substrates.

In this study, the two model populations (*Gordonia* and *Acinetobacter*) were further characterized in the same activated sludge system by determining the dynamic behavior of their respective rRNA pool under the typical diurnal COD loading rate of a municipal activated sludge reactor. A structured model of the biomass able to describe the dynamics of the RNA pool was then developed. Finally, the current modeling description of the substrate found in municipal wastewater (readily and slowly degradable substrate) was used in conjunction with the structured biomass model to simulate the observed increase in cellular RNA level.

Model development

Cell structure

A bacterial cell is composed of DNA, RNA, proteins, lipids, peptidoglycan, storage compounds (e.g. glycogen and polyhydroxybutyrate), metabolites, cofactors, and building blocks. For modeling purposes, these components can be grouped into four compartments based on cellular functions and chemical composition (Figure 1a). The first compartment comprises the metabolites, cofactors, and building blocks (X_B). In the model, this compartment is the metabolic pivotal point since it controls the production of storage and the composition of new biomass. The second compartment is the storage compartment (X_{STO}). This model structure is a simple extension of the activated sludge model no. 3 (ASM3; IWA Task Group on Mathematical Modeling, 2000).

The third and fourth compartments contain the active fraction of the cell biomass (X_A) and mediate the catalytic reactions of the cell. The third compartment comprises the protein synthesis system (PSS). This system carries the single most important catalytic reaction of the cell. Thus, a large fraction of the cell biomass (up to 60% at the fastest growth rates) is devoted to the PSS (Bremer and Dennis, 1996; Ingraham *et al.*, 1983). For the structured model, all the RNA of the cell is grouped in this compartment and approximately 80–85% consists of rRNA. The PSS is also composed of a variety of proteins such as ribosomal proteins, aminoacyl-transfer RNA synthetases, and RNA polymerase and accounts for up to 50% of the proteins at the highest growth rates (Ingraham *et al.*, 1983).

In order to follow the amount of rRNA in the cell the PSS compartment was divided in two. The RNA ($X_{PSS,R}$) and proteins ($X_{PSS,P}$). The composition of the PSS is relatively constant independent of the growth rate suggesting coupled regulatory mechanisms (Ingraham *et al.*, 1983). Thus, it was assumed that the proportion of RNA in the PSS at any time was α_R, such that $X_{PSS,R} = \alpha_R \cdot X_{PSS}$ and $X_{PSS,P} = (1-\alpha_R) \cdot X_{PSS}$. This is a simplifying assumption, which contrasts somewhat with the complexity of the system that regulates the production of PSS components. We present below the main mechanisms regulating the expression of ribosomal proteins; the reader is referred to a review by Nomura (1999) for more details. The ribosomal proteins inhibit their own expression rate. Thus, the rate of ribosome assembly controls the synthesis rate of ribosomal proteins. The coupling of the expression of ribosomal proteins to the assembly of the ribosome allows for the system to be entirely regulated by the production rate of rRNA. Therefore, the precursor rRNA (an intermediate

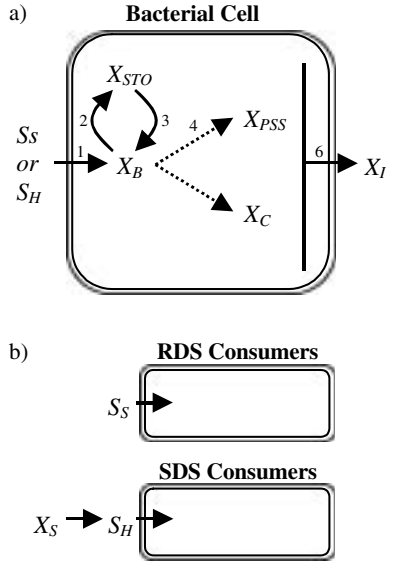

Figure 1 Flow of COD in the model. a) Flow of COD inside a bacterial cell (identical for RDS and SDS consumers). 1: substrate uptake, 2: storage accumulation, 3: storage breakdown, 4: Production of the PSS, 5: production of non-PSS cell components, 6: endogenous respiration converting X_C and X_{PSS} to X_I. The processes described by solid lines are mediated by the protein fraction of X_C and the processes described by the dotted lines are mediated by X_{PSS}. b) Flow of COD in the bacterial community. Two type of populations are assumed: RDS consumers and SDS consumers

molecule in the maturation pathway of rRNA) level increases faster than the rest of the PSS after a metabolic upshift breaking the equilibrium of the PSS components. The magnitude of the increase was, however, less than 1% of the total *Acinetobacter* RNA in a full-scale activated sludge system (Frigon *et al.*, 2001) validating the assumption that the PSS components are constantly in equilibrium.

The fourth compartment includes all other cell components not considered so far. This compartment was designated X_C. Proteins not involved the synthesis of new proteins are part of X_C. As opposed to the proteins of the PSS, the cellular level of this second group of proteins decreases with increasing growth rate (Ingraham *et al.*, 1983). Other cell components such as DNA, lipids, peptidoglycan are also included in this compartment. In order to properly fit the protein synthesis rate (see the section Cell processes), this compartment was subdivided in protein ($X_{C,P}$) and other components ($X_{C,L}$). The proportion of protein in X_C was defined as β_P, such that $X_{C,P} = \beta_P \cdot X_C$ and $X_{C,L} = (1-\beta_P) \cdot X_C$.

Cell processes

Six processes are considered to model the cellular reactions (Figure 1a, Table 1). The first process is substrate uptake, which is a first order reaction with respect to $X_{C,P}$ because the transporter proteins are grouped in this compartment. The next two processes are storage accumulation and breakdown. Again, these are first order reactions with respect to $X_{C,P}$. In this study, the emphasis was not on the dynamics of the storage pool, but on the dynamics of the RNA pool. We recognize, however, that the storage metabolism may affect the behavior of the RNA pool. For simplicity, the change in storage is modeled through an equilibrium reaction between the building block and storage compartments.

In this model, new active biomass is synthesized by the PSS using building blocks. Two processes are involved: production of PSS components and production of non-PSS components. In the first process, synthesis of X_{PSS} is a first order reaction with respect to the X_{PSS} itself. Data obtained with *Escherichia coli* show that the specific rate of synthesis of the PSS pools (RNA and proteins) is linear with the dilution rate of a chemostat (Bremer and Dennis, 1996). By assuming that the ratio between X_B and the sum of the active biomass components ($X_A = X_{PSS} + X_C$) follows a linear relationship with the growth rate, the PSS synthesis rate can be modeled using a first order relationship with this ratio. This is similar

Table 1 Stoichiometry and kinetic rate expressions matrix describing the structured cellular biomass. The same matrix applies to both RDS and SDS consumers

Components Processes	$S_{(S\text{ or }H)}$	S_o	X_B	X_{STO}	X_{PSS}	$X_{C,P}$	X_I	Process Rates
1) Substrate Uptake	$\frac{-1}{Y_U Y_G}$	$\frac{-(1-Y_U)}{Y_U Y_G}$	$\frac{1}{Y_G}$					$k_U \cdot \frac{S_{(S\text{ or }H)}}{K_U + S_{(S\text{ or }H)}} \cdot X_{C,P}$
2) Storage Accumulation			–1	1				$k_{STO} \cdot \frac{X_B}{X_A} \cdot X_{C,P}$ *
3) Storage Breakdown			1	–1				$k'_{STO} \cdot \frac{X_{STO}}{X_A} \cdot X_{C,P}$
4) Production of PSS		$\frac{-(1-Y_G)}{Y_G}$	$\frac{-1}{Y_G}$		1			$k_{PSS} \cdot \frac{X_B}{X_A} \cdot X_{PSS}$
5) Production of non-PSS Components		$\frac{-(1-Y_G)}{Y_G}$	$\frac{-1}{Y_G}$			1		$\left[\frac{k_C}{K_C + X_B/X_A} - (1-\alpha_R) \cdot k_{PSS}\right] \frac{X_B}{X_A} \cdot X_{PSS}$
6) Endogenous Respiration		$-(1-f_I)$			$\frac{-X_{PSS}}{X_A}$	$\frac{-X_C}{X_A}$	f_I	$b \cdot X_A$

*$X_A = X_{PSS} + X_C$

to the model proposed by Bleeken (1989), who suggested to use the X_B:X_A ratio to describe the transcription initiation activity for the PSS genes. This suggestion was recently substantiated experimentally for *E. coli* (Gaal *et al*., 1997).

The synthesis rate of X_C was determined by observing the total protein ($X_P = X_{PSS,P} + X_{C,P}$) synthesis rate. For *E. coli*, the synthesis rate of total protein follows a squared hyperbola (monod-type saturation function) with respect to the dilution rate of a chemostat. Assuming again that the X_B:X_A ratio increases linearly with the growth rate, the total protein synthesis rate can be described by a squared hyperbola with respect to the X_B:X_A ratio (Bleeken, 1989). This relationship can be explained by the accumulation of a larger PSS than needed by bacterial cells for the synthesis of proteins at lower growth rates (Koch, 1971). Part of the total protein synthesis rate is, however, already taken into account by the synthesis rate of the PSS. The synthesis rate of $X_{C,P}$ was therefore described as the difference between the total protein synthesis rate and the synthesis rate of the PSS proteins. For *E. coli*, this provided a good fit at all growth rates examined (data not shown). Finally, decay of the active biomass compartments to an inert compartment (X_I) is included in the model.

Community structure and processes

In contrast to the current ASM models, which assume that all the COD is consumed by all members of the microbial community, in this study the community is assumed to consist of two populations. One population consumes readily degradable substrates (RDS), and the other one uses slowly degradable substrates (SDS; Figure 1b). This model structure is similar to the one proposed in the early 1980s by the group of the University of Cape Town (South Africa) and was later abandoned in the formulation of ASM1 (Dold and Marais, 1986).

The consumption of SDS essentially is described as in ASM3 except that hydrolysis is a first order relationship with the size of the non-PSS protein compartment ($X_{C,P}$) of the population consuming SDS. The substrate produced from hydrolysis (S_H) is also taken up entirely by the population consuming SDS. The structure of the cell is the same for both populations.

Materials and methods

Reactor sampling

The activated sludge system of the Urbana-Champaign Sanitary District Northeast (UCSD-NE) wastewater treatment plant (Urbana, IL, USA) is a contact-stabilization system consisting of four aeration tanks and four secondary clarifiers. During the study, the system was operated at a hydraulic retention time (HRT) of six to eight hours and a solids retention time (SRT) of approximately four days. The influent to the activated sludge reactor (primary effluent) was sampled every two hours for a period of three weeks to determine the COD loading rate. The samples were acidified to pH 2 with sulfuric acid and kept at 4°C until analysis. COD concentrations were determined using Hach vials according to the manufacturer's instruction (Hach, Loveland, CO, USA). The biomass from each tank was sampled at 6:00 h, 12:00 h, and 18:00 h every four days during the three-week study period. Biomass samples were frozen at –80°C within two hours after sampling.

Molecular analysis

The RNA from approximately 15 mg of suspended solids was extracted by a hot phenol-bead beating procedure (Raskin *et al*., 1995; Stahl *et al*., 1988). RNA was denatured with 2% glutaraldehyde for 10 min and applied to a Magnacharge nylon membrane (Osmonics Inc., Minnetonka, MN, USA; Raskin *et al*., 1994). The membranes were hybridized

overnight with ^{32}P-labeled oligonucleotide probes at 40°C using the Sigma "Perfect HYB" buffer (Sigma, St. Louis, MO), washed twice for one hour at the same temperature using a sodium dodecyl sulfate-sodium citrate buffer, and washed for 30 min at a specific temperature (Raskin *et al*., 1994). The probes used in this study target: *Acinetobacter* spp. (name: S-G-Acin-0659-a-A-24, sequence: CTGGAATTCTACCATCCTCTCCCA, wash temperature: 64ºC; Oerther *et al*., in prep.), *Gordonia* spp. (name: S-G-Gor-0596-a-A-22, sequence: TGCAGAATTTCACAGACGACGC, wash temperature: 54ºC; de los Reyes *et al*., 1997), and virtually all organisms (name: S-*-Univ-1390-a-A-18, sequence: GACGGGCGGTGTGTACAA, wash temperature: 44ºC; Zheng *et al*., 1996). The signal obtained with each specific probe was normalized using the signal obtained with probe S-*-Univ-1390-a-A-18. To ensure a normal distribution, the ratio data were transformed using the arcsine-square root transform (Sokal and Rohlf, 1995). Subsequent calculations such as averages were calculated with the transformed data. The population data were statistically analyzed by repeated measures of analysis of variance. A significance level (α) of 0.10 was adopted for hypothesis testing.

Modeling

The parameters of the structured biomass model were determined using steady-state data for *E. coli* growing in a glucose-fed chemostat at 37ºC (Bremer and Dennis, 1996). All the rate constants were assumed to vary similarly with respect to temperature following the temperature relationship suggested for the ASM3 model (Table 2, IWA Task Group on Mathematical Modeling, 2000; Koch *et al*., 2000). The value of the parameters involved in hydrolysis and decay were the same as in Koch *et al*. (2000). The values of all parameters used for model simulations are reported in Table 2.

The X_S:S_S ratio in the reactor influent was set to 11:2 which is comparable to Koch *et al*. (2000) who modeled a full-scale wastewater treatment plant. The profiles used for simulation of diurnal variations in influent COD concentration and flow rates are shown in Figure 2.

Table 2 Values of the parameters used in the model simulations

Description	Symbol	Units	Value (20EC)*
Hydrolysis rate constants	k_H	g X_S g^{-1} $X_{C,P,XS}$ d^{-1}	9.0**
Hydrolysis half-saturation constant	K_H	g X_S g^{-1} $X_{C,P,XS}$	1.0**
Substrate uptake rate constant	k_U	g (S_S or S_H) g^{-1} $X_{C,P}$ d^{-1}	92.0
Substrate uptake half-saturation constant	K_U	g (S_S or S_H) m^{-3}	1.0
Storage accumulation rate constant	k_{STO}	g X_B g^{-1} $X_{C,P}$ d^{-1}	167.8
Storage breakdown rate constant	k'_{STO}	g X_{STO} g^{-1} $X_{C,P}$ d^{-1}	167.8
PSS synthesis rate constant	k_{PSS}	g X_{PSS} g^{-1} X_{PSS} d^{-1}	167.8
Non-PSS cellular components synthesis rate constant	k_C	g $X_{C,P}$ g^{-1} X_{PSS} d^{-1}	23.0
Non-PSS cellular components synthesis half-saturation constant	K_C	g X_B g^{-1} X_A d^{-1}	0.02
Endogenous respiration rate	b	d^{-1}	0.3**
Building block yield by substrate uptake	Y_U	g X_B g^{-1} (S_S or S_H)	0.8**
Active biomass yield per building block	Y_G	g X_A g^{-1} X_B	0.8**
Non degradable fraction of active biomass	f_I	g X_I g^{-1} X_A	0.2**
Fraction of RNA in PSS	α_R	g $X_{PSS,R}$ g^{-1} X_{PSS}	0.48
Fraction of proteins in non-PSS cell components	β_P	g $X_{C,P}$ g^{-1} X_C	0.73
Solids retention time	SRT	d	4.0
Hydraulic retention time	HRT	d	0.25
Recycling flow		% of influent flow	30.0

* Only the rate constants were consider to vary with temperature. The temperature dependency was assumed to be $k_T = k_{20°C} \cdot \mathbf{exp}((\theta \cdot (\mathbf{T - 20°C}))$ with $\theta_T = 0.04$ for the hydrolysis rate constant and $\theta_T = 0.07$ for the other rate constants

** These values were taken from a calibration of the ASM3 model (Koch *et al*., 2000)

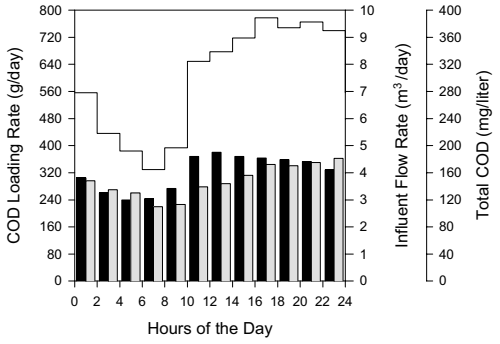

Figure 2 Diurnal variation of total COD loading rate (——), flow rate ( ), and total COD concentration ( ) of the activated sludge reactor influent as used for model simulations. The reactor volume was set to 1 m^3 during the simulations. The relative flow rates and COD loading rates are similar to the UCSD-NE treatment plant during the time of the three-week study

Results and discussion

Dynamics of rRNA under diurnal loading

In this section, data obtained with samples from the UCSD-NE wastewater treatment plant on the diurnal dynamics of the rRNA pool of *Acinetobacter* spp. and *Gordonia* spp. are reported. This study was published in more detail elsewhere (Frigon *et al.*, 2001), but the data are presented here to allow comparison with modeling results. The UCSD-NE wastewater treatment plant was studied for three weeks. During this period, the COD loading rate (both the COD concentration and the influent flow rate) was minimum around 6:00 h, increased rapidly through the morning to reach a maximum around noon (12:00 h), stayed relatively stable until midnight (24:00 h), and decreased through the night (Frigon *et al.*, 2001).

During the same study period, eight mixed liquor samples were taken along the length of the reactor at 6:00 h, 12:00 h, and 18:00 h every four days. Total RNA was extracted and the rRNA concentrations of *Acinetobacter* and *Gordonia* were determined by oligonucleotide probe membrane hybridization. The total level of rRNA did not change during the day, nor did it change along the length of the reactor (data not shown; $P>0.10$). Differences were observed in the dynamics of rRNA of the two model populations. While the rRNA level of *Gordonia* remained constant (Figure 3a; $P>0.10$), the rRNA level of *Acinetobacter* increased over a one day time period (Figure 3b; $P<0.10$). These data agree with our previous results suggesting the presence of two types of bacterial populations in municipal activated sludge systems (Oerther *et al.*, in prep.; Oerther *et al.*, 2001). A possible mechanism for the presence of bacterial populations with different RNA pool dynamics is that the populations have different functions in the community. In this scenario, *Acinetobacter* would be specialized in utilizing RDS and *Gordonia* would be specialized in utilizing SDS. The mathematical model describes the possibility that, under this hypothesis, the degradation rates of the substrates determine the dynamics of the RNA pools.

Simulation of RNA pools

In search of an interpretation for the presence of two population types (high RNA-low biomass and low RNA-high biomass content population) in municipal activated sludge systems, we decided to develop a simple model describing the dynamics of the rRNA pool. The structured model of bacterial cells developed by Bleeken (1989) was extended to include two protein compartments (PSS and non-PSS proteins) and a storage compartment. The values of the parameters of the cellular processes were determined based on the

steady-state cellular composition of *E. coli* growing in a glucose-fed chemostat (Bremer and Dennis, 1996). The current description of substrate found in municipal wastewater (RDS and SDS; IWA Task Group on Mathematical Modeling, 2000) was used to implement the structured model of the cell. It was assumed, however, that the RDS and SDS were consumed by two different populations.

A series of simulations were run to evaluate the effect of reactor configuration, type of substrate, and storage metabolism on the level of RNA in the biomass. The model predicts that the cellular RNA level is the same for the population consuming SDS at all conditions (Table 3). The situation is different for the population consuming RDS. This population has the same level of RNA as the population consuming SDS when grown in a completely mixed stirred tank reactor (CSTR). On the other hand, the average RNA level of the population consuming RDS is higher than the RNA level of the population consuming SDS in a plug-flow reactor (PFR; Table 3). The incorporation of storage metabolism in the model slightly reduces the predicted level of RNA for the population consuming RDS in a PFR (Table 3) by reducing the peak in X_B during the feast period at the beginning of the PFR cycle.

When the reactor influent flow rate and composition are constant, CSTR theory predicts a constant substrate level at all times. This explains why the cellular rRNA level is the same for both populations independent of the substrate type. The main parameter affecting the rRNA level is the SRT (data not shown). These modeling results are consistent with chemostat experiments showing that the cells reach a steady-state composition determined by the growth rate and not by the substrate utilized (Ingraham *et al*., 1983; Maaløe and Kjeldgaard, 1966).

It has been demonstrated experimentally that cyclical feeding of a chemostat operated with a 10-hour feeding cycle forces bacterial cells to accumulate more RNA than cells growing in a chemostat fed at a constant rate (Sepers, 1986). This situation is analogous to conditions in a PFR in which the cells experience a substrate gradient along the length of the reactor. Our model predicts that the RNA level of the population consuming RDS is higher than what is necessary to double the biomass at the SRT when the cells are growing in a PFR (Table 3), a result in agreement with the experimental data. From a metabolic point of view, the phenomenon can be understood as follow. As the cells encounter high substrate concentrations, they will grow fast until they deplete the substrate. This corresponds to a metabolic upshift. Adapting to this high growth rate, the cells primarily produce rRNA and the other components of the PSS (Ingraham *et al*., 1983; Maaløe and Kjeldgaard, 1966). The cells start producing the other cell components only when the cellular rRNA reaches the level corresponding to the high growth rate. Typically, the cell reaches the new cellular RNA level within a few minutes after a metabolic upshift (Ingraham *et al*., 1983; Maaløe and Kjeldgaard, 1966). On the other hand, the cell does not actively degrade the extra rRNA when it undergoes a metabolic downshift to reduce its growth rate. In a metabolic downshift, the synthesis of new rRNA is reduced while the synthesis of the rest of the cell components is maintained at a high rate until the cellular rRNA reaches the lower level by dilution in the newly synthesized cell components (Ingraham *et al*., 1983; Maaløe and Kjeldgaard, 1966). Thus, if the cell stops growing abruptly after a period of high growth rate, the RNA level stays high for a certain period of time (Herbert, 1961). This scenario is properly decribed by the model presented here.

The situation is different for cells growing on SDS in a PFR. In this case, the cells do not experience a feast-famine cycle because the growth is always limited by the rate of hydrolysis of the substrate. Furthermore, the parameter affecting the level of RNA is the SRT. Therefore, the cellular RNA for the population consuming SDS is the same in a CSTR and in a PFR if the SRT is the same for both reactors.

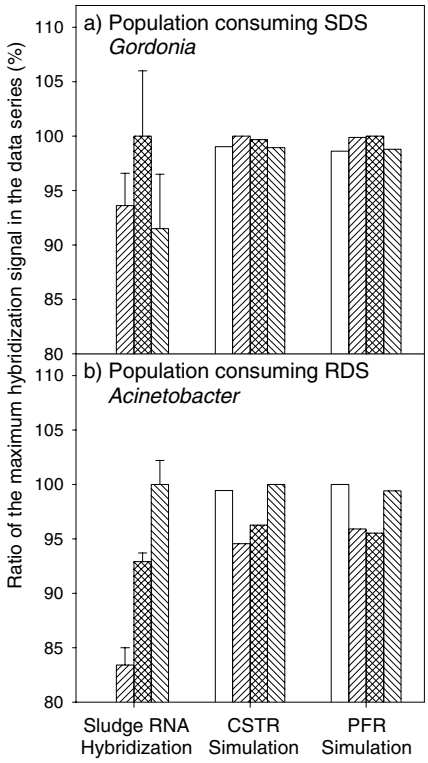

Figure 3 Diurnal dynamics of the RNA pool for the bacterial populations consuming SDS (a) and RDS (b) in a municipal activated sludge wastewater treatment plant under variation of the COD loading rate. For hybridization of sludge RNA, the population consuming SDS was *Gordonia* and the population consuming RDS was *Acinetobacter*. Model simulations for the corresponding populations are presented in the same panel. Each bar represents a sampling time: 0:00 h (□), 6:00 h(▨), 12:00 h (▩), 18:00 h (▧). Error bars are the average standard deviations (n = 8 samples per day) of 7 sampling days

Impact of the storage metabolism

In a PFR, the cells consuming RDS experience a feast-famine cycle due to the gradient of substrates along the length of the reactor. Thus, the cells produce new biomass at a high rate during the feast period at the beginning of the reactor, and at a much lower rate during the famine period. The active biomass production rate during the feast period was reported to be as high as six times 1/SRT for cells growing in sequencing batch reactors (SBR) operated at a SRT of approximately 4 days and fed glucose or acetate (Beun *et al.*, 2000; Dircks *et al.*, 2001). During the feast period, the cells store a large portion of the substrate they take up, but they also synthesize as much as 34% of the active biomass produced during the feast-famine cycle (Beun *et al.*, 2000). As the biomass production rate is high during the feast period, the cells will presumably try to synthesize high levels of RNA to accommodate the biomass production rate. Thus, the cells should accumulate a higher level of RNA than what is required by the SRT as the model presented here predicts.

It was suggested that the storage metabolism changes the dynamics of active biomass synthesis by equalizing the growth over the feast-famine cycle (Beun *et al.*, 2000; Dircks *et al.*, 2001). This would reduce the level of RNA accumulated during the feast-period as seen in the model simulation results (Table 3). Inspection of the storage kinetic data reported in the literature suggests, however, that the relationship between specific substrate compounds, storage metabolism, and biomass synthesis metabolism is crucial in determining

Table 3 Model simulation results of the average level of RNA in bacterial biomass for the populations consuming RDS and SDS

Population	Modeling Conditions	CSTR	PFR
PFR			
		(% g XPSS,R g–1 XT)*	
SDS Consumers	Constant COD loading		
	With Storage Metabolism	6.40	6.41
	Without Storage Metabolism	6.42	6.44
	COD loading varying diurnally		
	With Storage Metabolism	6.46	6.47
	Without Storage Metabolism	6.48	6.49
RDS Consumers	Constant COD loading		
	With Storage Metabolism	6.40	10.37
	Without Storage Metabolism	6.42	11.94
	COD loading varying diurnally		
	With Storage Metabolism	6.58	10.87
	Without Storage Metabolism	6.60	12.68

* $X_T = X_B + X_C + X_{PSS} + X_{STO}$

the effect of storage on biomass synthesis. In the same SBR experiments as discussed above, only 7.5% of the biomass was synthesized during the feast period when glucose (stored as glycogen) was used as substrate (Dircks *et al.*, 2001), while 34% of the biomass was synthesized during the feast period when acetate (stored as polyhydroxybutyrate) was the substrate (Beun *et al.*, 2000). It is not clear how this difference affects the cellular RNA level.

Structure of the bacterial community

By simply assuming that RDS and SDS are consumed by two different populations, it was possible to simulate the behavior of the RNA pool of *Acinetobacter* spp. and *Gordonia* spp. The model describes that the population consuming SDS has a low RNA level per biomass in all reactor configurations (Table 3) and the RNA level remains constant despite a diurnal variation of the COD loading rate (Figure 3a). The population of *Gordonia* spp. corresponds to this description (Figure 3a and Oerther *et al.*, 2001). The model further describes that the RDS has a high RNA level per biomass in a PFR (Table 3) and that the RNA level varies diurnally with the COD loading rate (Figure 3b). The population of *Acinetobacter* spp. exhibits these characteristics (Figure 3b and Oerther *et al.*, in prep.). Thus, it is suggested that bacterial populations growing in an activated sludge treatment system specialize in consuming either RDS or SDR.

The division of the microbial community into two populations was considered 20 years ago during the development of ASM1, but was rejected for simplicity (Dold and Marais, 1986). The molecular data seem to support the two-population model structure. This understanding of the community as consisting of two bacterial populations may be useful in modeling situations involving several populations such as bulking and foaming. The simple identification of substrate degradation kinetics would allow modelers to properly describe the growth dynamics of the populations present in the system. The data presented here suggest that the kinetics of substrate degradation could be determined for each population by characterizing (i) the level of RNA per biomass, and (ii) the dynamics of the RNA pool under diurnal loading. Thus, a natural link may exist between the molecular identification of populations causing problems in activated sludge, and the description of the growth of these populations by means of mathematical modeling.

Conclusions

- Two types of populations with different RNA pool dynamics were identified in activated sludge wastewater treatment systems. The first population contains a high level of RNA per biomass and its RNA level varies diurnally following the COD loading rate. The second population contains a low level of RNA per biomass and its RNA level remains stable despite the diurnal variation in COD loading rate.
- The two types of populations identified on the basis of the dynamics of the RNA pool are suggested to have different functions in the activated sludge community. Populations with a high level of RNA per biomass consume readily degradable substrates (RDS), while populations with a low level of RNA per biomass consume slowly degradable substrates (SDS).
- The level of RNA in the population consuming RDS is affected by the storage metabolism. The relationship between specific substrate compounds, storage metabolism, and biomass synthesis metabolism may be important in determining the impact of the storage metabolism on the level of RNA. Further work is required to clarify this relationship.

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