Transient Response of Aerobic and Anoxic Activated Sludge Activities to Sudden Substrate Concentration Changes

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Abstract: The state-of-the-art understanding of activated sludge processes as summarized in activated sludge models (ASMs) predicts an instantaneous increase in the biomass activity (which is measured, e.g., by the corresponding respiration rate OUR, NUR, etc.) under sudden substrate concentration changes. Experimental data (e.g., short-term batch respiration experiments under aerobic or anoxic conditions) collected for the calibration of the dynamic models (ASMs) often exhibit a transient phenomenon while attaining maximum activity, which cannot be explained by the current understanding of the activated sludge process. That transient phenomenon exhibits itself immediately upon addition of a substrate source to an endogenously respiring activated sludge sample and it usually takes a few minutes until the activated sludge reaches its maximum possible rate under given environmental conditions. This discrepancy between the state-of-the-art model and the experimental data is addressed in detail in this investigation. It is shown that the discrepancy is not caused by an error in the experimental set-up/data but it is rather due to model inadequacy. Among the hypotheses proposed, it appears that this transient response of the activated sludge most likely results from the sequence of intracellular reactions involved in substrate degradation by the activated sludge. Results from studies performed elsewhere with pure cultures (*S. cerevisae* and *E. coli*) support the hypothesis. The transient phenomenon can be described by a dynamic metabolic network model or by a simple first-order model, as adopted in this study. The transient phenomenon occurring in short-term batch respiration experiments is shown to interfere severely with parameter estimation if not modeled properly (2.8%, 11.5%, and 16.8% relative errors [average of three experiments] on Y_H, μ_{maxH}, and K_S, respectively). Proper modeling of this transient phenomenon whose time constant is on the order of minutes (1 to 3 min) is expected to contribute fundamentally to a better understanding and modeling of Orbal, carousel, and SBR-type treatment plants with fast-alternating process conditions, although such studies are beyond the scope of this report. © 2004 Wiley Periodicals, Inc.

Keywords: short-term batch experiments; activated sludge modeling; parameter estimation; respirometry; substrate metabolism; titrimetry; alternating systems; SBR

INTRODUCTION

Short-term batch substrate degradation experiments, usually initiated by pulse substrate additions to activated sludge previously sampled from a full-scale plant, are often used in the framework of full-scale wastewater treatment plant (WWTP) model calibrations. Such experiments produce data that are sufficiently informative for estimating kinetic and/or stoichiometric parameters of the activated sludge (Vanrolleghem et al., 1995, 1999). Biomass oxygen uptake rates (OURs), ammonium uptake rates (AURs), nitrate uptake rates (NURs), or biomass proton production rates (HpRs) are examples of data sets that most often result from the batch experiments. An overview of available methodologies for data collection can be found in studies by Vanrolleghem et al. (1999) and Petersen et al. (2003). The data, representing the response of the activated sludge sample to a pulse substrate addition, are often interpreted using a model; that is, the model is fitted to the data by modifying the values of kinetic and/or stoichiometric model parameters. The resulting parameters are subsequently transferred to the full-scale plant model and combined with others (Petersen et al., 2003).

In this study, it will first be shown that the experimental observations during a short-term batch substrate degradation experiment after pulse substrate addition do not correspond to the expectations based on the state-of-the-art activated sludge models. A transient initial response is often observed in the experimental data that is not accounted for by the activated sludge models. The main objective of this study is to investigate, understand, and mathematically describe the underlying mechanisms for this experimentally observed

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transient response, because it bears a major limitation regarding the applicability of short-term batch experiments with activated sludge in dynamic model calibration procedures. After describing the nature of the observed phenomena, several hypotheses are formulated to explain the experimental observations. In view of the experimental results, the importance of proper understanding and modeling of the dynamic transient response phenomenon will be highlighted not only for interpretation of the short-term calibration experiments but also for modeling the full-scale WWTP.

PROBLEM STATEMENT

Note that, throughout this study, the term ''batch experiment'' is used to indicate short-term (e.g., between 15 and 60 min) batch substrate degradation experiments with activated sludge following a pulse substrate addition. The state-of-the-art activated sludge models and typical experimental data obtained from batch experiments are introduced. The problem statement originates from the discrepancies of these models with the data.

Expected Activated Sludge Response to Sudden Concentration Changes: State-of-the-Art Description

Simulation of full-scale WWTPs as well as interpretation of experimental results from batch experiments is based on state-of-the-art understanding of activated sludge behavior, which is summarized in ASMs (Henze et al., 2000). For a batch experiment with a pulse substrate addition, the models (Table I) predict an instantaneous increase in the biomass activity (Fig. 1). The activated sludge is in endogenous state at $t = 0$ and, according to the model, the biomass reacts to pulse substrate addition with an immediate increase in the OUR to a maximum level of 0.83 mg O_2/L·min, indicating that the substrate is immediately metabolized at the maximum rate.

Experimental Observation of Activated Sludge Response to Sudden Concentration Changes: Reality

A clear discrepancy between model predictions (Fig. 1) and the experimental response obtained in a batch experiment can be observed during the first minutes of the experiment. Indeed, the OUR in Figure 2 shows a clear transient response after pulse acetate addition. Note that the transient response to a pulse substrate addition can be observed only on the condition that the data acquisition frequency is on the order of seconds. According to Orhon et al. (1995), for example, an OUR sampling interval of 10 min did not allow observation of the transient phenomenon in the OUR profiles, although a similar experimental design was used as for the experiment in Figure 2.

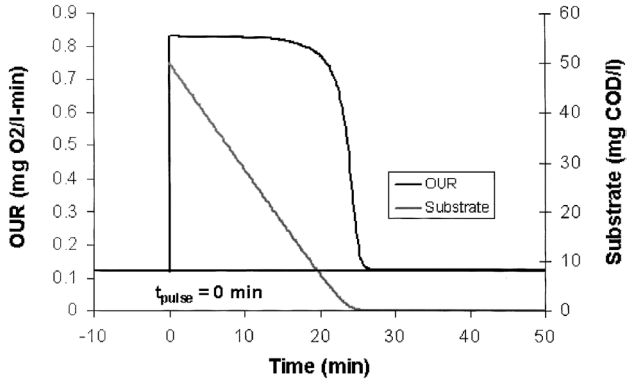

Figure 1. Simulation of activated sludge response to a pulse addition of readily biodegradable substrate in a batch reactor based on ASM1 (Henze et al., 2000). The following parameters were used in the simulation: $S_S(0) = 50$ mg COD/L; $Y_H = 0.67$ mg COD/mg COD, $\mu_{maxH} \cdot X_H = 1.5$ mg COD/L · min, $K_S = 1$ mg COD/L, and $b_H = 0.00015$ 1/min.

Discrepancy Between Model and Data Is a More General Phenomenon

The observation of the transient response during batch experiments is not limited to the data presented in this study. In many cases, published data show similar phenomena (Ficara et al., 2000; Kong et al., 1996; Ning et al., 2000; Spanjers and Vanrolleghem, 1995; Spérandio and Paul, 1997; Strotmann et al., 1999). In these studies, however, the transient phenomenon did not receive any attention and was neglected during interpretation of the data. In some studies, the investigators(s) apparently preferred to present only that particular part of the OUR data that can be explained with Monod kinetics (e.g., Vanrolleghem et al., 1995), whereas the initial part of the data, including the transient phenomenon, was not shown.

Observations of the transient phenomenon are not limited to heterotrophic substrate degradation processes under aerobic conditions. The transient response was also observed for respirometric experiments with nitrifying biomass (Ficara et al., 2000; Gernaey et al., 2001; Spanjers and Vanrolle-

Table I. Matrix representation of the mathematical model used to interpret respirometric data resulting from pulse addition of substrate to an endogenously respiring activated sludge sample.

Process	Component					Process rate
	1. S_S	2. S_O	3. S_{NH}	4. X_A	5. X_H	
1. Growth of heterotrophs	$-\frac{1}{Y_H}$	$-\frac{1-Y_H}{Y_H}$	$-i_{XB}$		1	$\mu_{maxH}(1-e^{-t/\tau_H})\frac{S_S}{S_S+K_S}X_H$
2. Growth of nitrifiers		$-\frac{4.57-Y_A}{Y_A}$	$-i_{XB}-\frac{1}{Y_A}$	1		$\mu_{maxA}(1-e^{-t/\tau_A})\frac{S_{NH}}{S_{NH}+K_{NH}}X_A$

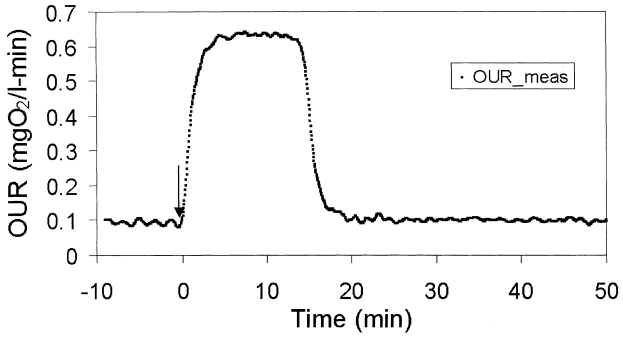

Figure 2. Typical activated sludge response to acetate addition (30.32 mg COD/L acetate) in batch experiments. The maximum OUR is reached after a transient period.

ghem, 1995). Furthermore, Sin et al. (2003) observed the transient phenomenon for anoxic experiments with heterotrophic biomass.

Observing the transient phenomenon is not limited to OUR data based on dissolved oxygen electrodes. OUR data can also be extracted from off-gas oxygen measurements. Pratt et al. (2002) argued that the transient phenomenon in their data might be due to the lag time of the off-gas measurement system, but did not test this hypothesis in detail. A transient phenomenon was also present in the OUR profiles reported by Tusseau-Vuillemin et al. (2002), who measured the OUR using two different respirometric methods: titrimetric addition of H_2O_2 vs. aeration in batch reactors. Again, no assessment of the behavior was conducted.

The examples suggest that the transient phenomenon occurs in every experiment with pulse substrate additions to activated sludge. The measurement frequency during respirometric measurements is very important, because the transient phenomenon is fast, with a time constant on the order of 1 to 3 min. Thus, although it is expected that the ransient response occurs in most respirometric assays, it can only be detected in set-ups with a sufficiently high measurement frequency.

MATERIALS AND METHODS

Most of the data presented in this study were obtained using the RODTOX sensor (Vanrolleghem et al., 1994). The RODTOX (Kelma bvba, Niel, Belgium) is a batch respirometer. The reactor vessel, filled with 10 L of activated sludge, is constantly aerated, stirred, and thermostatted. In the cover of the bioreactor, dissolved oxygen (DO) and pH probes are installed. Two different types of Endress and Hauser DO electrodes (Conducta 905 and Conducta 905 S) were used during the investigation.

Some data were obtained with the combined respirometric–titrimetric sensor (Gernaey et al., 2001), which allows measurement of combined OUR and HpR data. Data sets under anoxic conditions were collected using the integrated sensor of Sin et al. (2003), which allows measurement of combined NUR and HpR data. An explanation of the operating principles of these sensors is not essential for the present investigation. Basically, both sensors allow for acquisition of data similar to those of the RODTOX sensor.

Simulations were performed using WEST (Hemmis NV, Kortrijk, Belgium), dedicated software for WWTP modeling (Vanhooren et al., 2003). The mathematical model is based on the ASM1 model structure (Henze et al., 2000), unless explicitly specified otherwise.

RESULTS

The experimental set-up, a physicochemical phenomenon, or an insufficient description of the biological response with the model used for interpretation of the experimental data could all be reasons for the observed discrepancy between model predictions (Fig. 1) and experimental observations obtained from batch experiments (Fig. 2). In what follows, we attempt to ascertain whether the observed discrepancy is due to an error or misinterpretation of the experimental data, a physicochemical phenomenon, or a modeling error.

Analysis of Experimental Set-Up and Data

The following properties of the experimental set-up may contribute significantly to the observed transient phenomenon (Fig. 2): (a) the dynamics of the DO electrodes; or (b) the mixing characteristics of the experimental set-up. Each of these hypothetical explanations of the transient is assessed within the framework of its potential contribution to the observed discrepancy between model predictions and experimental data.

Dynamics of the Dissolved Oxygen Electrode

The dynamics of DO electrodes have been described most often as a first-order process with time constants of between 5 and 100 seconds (Lee and Tsao, 1979). Knowing the DO electrode model [Eq. (1)] and its experimentally determined parameter, τ, the actual DO concentration, S_O, can be retrieved from the electrode output, E:

$$\frac{dE}{dt} = \frac{1}{\tau}(S_O - E) \tag{1}$$

where S_O is the true DO concentration in the liquid phase (mg O_2/L), E is the DO concentration measured by the DO electrode, and τ is the experimentally determined first-order time constant of the DO electrode. Up-step responses of the DO electrode have been recorded by applying an experimental approach (Philichi and Stenstrom, 1989). The down-step response of the DO electrodes was measured in the same set-up, but this time the electrode was removed from the reactor containing water saturated with oxygen and inserted into deoxygenated water. Figure 3A shows the response (both up-step and down-step) for the Conducta 905 electrode. Because both S_O and E are known in the experiment, the data allow determination of the first-order

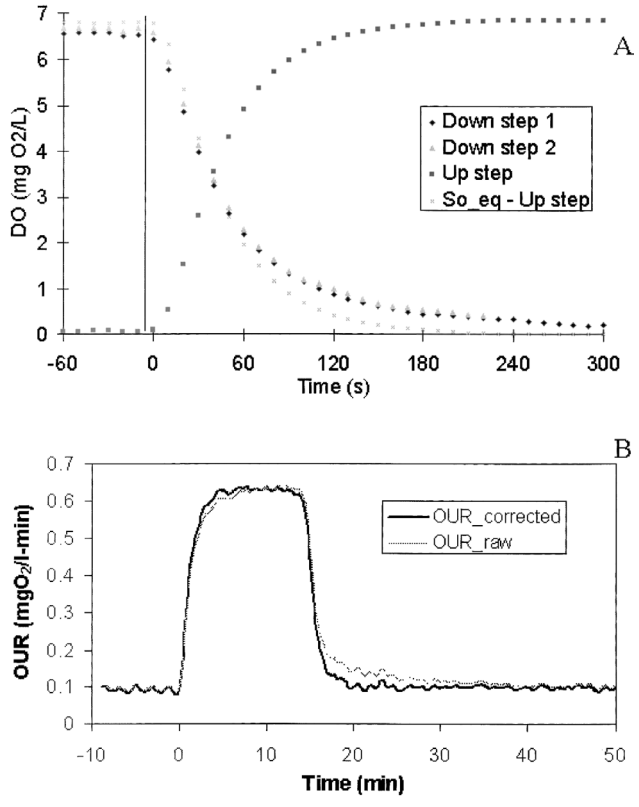

Figure 3. Typical up- and down-step responses of DO electrodes (Conducta 905, E+H) (A) Comparison of OUR corrected (solid line) and OUR_raw not corrected (dashed line) for the response time constant of the DO probe dynamics (B).

time constant, τ [see Eq. (1)]. For the Conducta 905 electrode, the value of τ was 55 s, whereas for the Conducta 905 S it was 12.5 s.

It is apparent that the time constant of DO electrodes has to be taken into account to appropriately model the dynamics of batch experiments. In addition, attention must be paid to noise elimination (Sin et al., 2003), because taking derivatives during the OUR calculation enhances the effect of noise. Applying this probe model [Eq. (1)], it was found that the electrode dynamics could only partially explain the transient phenomenon (see Fig. 3B). Yet, it is important to stress that the DO probe dynamics should be taken into account properly [e.g., by applying Eq. (1)] while calculating the OUR in any experimental set-up. In this study, the OUR values were always corrected for the response time of the DO probes.

Mixing Characteristics in Batch Reactors (Verification of CSTR Assumption)

The transient phenomenon in the data (Fig. 2) could be the consequence of insufficient mixing. A colorimetric method was used to evaluate the mixing characteristics of the reactor vessel. Phenolphthalein was added to the RODTOX vessel filled with 10 L of water. Addition of an excess base causes the color of the phenolphthalein to immediately change from colorless to violet. Samples were taken automatically every 2 s in the reactor at a location close to the DO electrode. The color intensity was analyzed using spectrophotometry. Two series of mixing experiments were performed: one series with stirring and aeration on (aeration intensity 15 L/min), and another series with stirring but no aeration (Fig. 4). The degree of mixing, m, is used in this study to quantitatively describe the tracer experiments (Nielsen et al., 2003):

$$m = \frac{s(t) - s(0)}{s_\infty - s(0)} \tag{2}$$

where $s(t)$ is the concentration of the tracer at time t (in this case it is the phenolphthalein concentration measured indirectly as color intensity), $s(0)$ is the initial concentration of the tracer, and s_∞ is the concentration as time approaches infinity where m becomes 1. The mixing time, t_m, is defined as the time needed to reach a value of m = 95%. The mixing time is about 10 s in the case of stirring only. With aeration the mixing is very fast, as the sample taken after 2 s already shows the final concentration. Hence, it can be concluded that mixing of the substrate injected in the aerated batch reactor occurred significantly faster than the transient phenomenon observed in the experimental data. Thus, improper mixing does not contribute significantly to the transient phenomenon.

Physicochemical Phenomena: Diffusion of Substrate Into Activated Sludge Flocs

Activated sludge grows in flocs. The flocs are simply clumps of bacteria that stick together (e.g., due to the presence of significant amounts of exopolymers). Diffusion phenomena around the activated sludge flocs might be a determining factor in the transient phenomenon. This hypothesis can be qualitatively described as follows: Just before pulse substrate addition, the sludge flocs can be assumed fully penetrated with oxygen and lacking substrate. When a substrate pulse is added to the bulk liquid, substrate diffuses into the

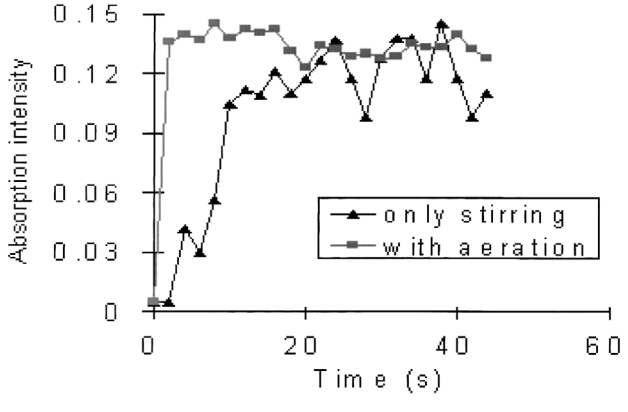

Figure 4. Absorption intensity measured at the position of the DO electrode for a mixing experiment with stirring only, and an experiment with stirring and aeration.

activated sludge flocs where aerobic microorganisms oxidize it, thereby consuming a significant amount of oxygen. This induces a difference in oxygen concentration between the flocs and the bulk liquid phase, and oxygen diffuses from the bulk liquid phase into the flocs. Finally, the decrease in oxygen concentration in the bulk is measured by the DO electrode. This would mean that the respirometric response to a pulse substrate addition may be influenced by diffusion limitations of either substrate or oxygen, or both.

To evaluate diffusion limitations, the following empirical approach was employed. An experiment was set up in which the respiration response of a single, nonflocculating *Pseudomonas* culture was compared with the response of an activated sludge sample. In the *Pseudomonas* culture, no diffusion limitations for substrate or oxygen can exist as the cells do not flocculate. Addition of an acetate pulse to the *Pseudomonas* culture resulted in the respirogram shown in Figure 5. The time between substrate addition and measurement of the maximum OUR was still 3 to 4 min. It can be concluded that the hypothesis of diffusion limitation in the sludge flocs cannot adequately explain the transient behavior observed in microbial cultures following pulse substrate addition. The behavior appears to be a characteristic of the bacterial cell.

The experiment with the *Pseudomonas* culture showed that the transient phenomenon could not be explained by transport limitations in the flocs. In support of this result, Li and Ganczarczyk (1992) stated that sludge flocs may be permeable, depending on their size and the plant operating conditions. They also suggested that advective transport through channels in the sludge flocs could be the main mass transfer mechanism, which could explain the absence of transport limitations in the flocs.

Summary: Evaluation of the Hypotheses

The three hypotheses just given offer only a partial explanation of the observed discrepancy between model predictions and the experimental data. Thus, the hypothesis that the experimental set-up or that physicochemical phenomena cause the discrepancy has to be rejected in favor of the alternative hypothesis: *The discrepancy is caused to a large extent by a modeling error*. A final argument to support the thesis that the transient phenomenon is not due to the experimental set-up used in our work is that the transient response phenomenon has also been observed in many different experimental set-ups, as indicated in the aforementioned literature survey.

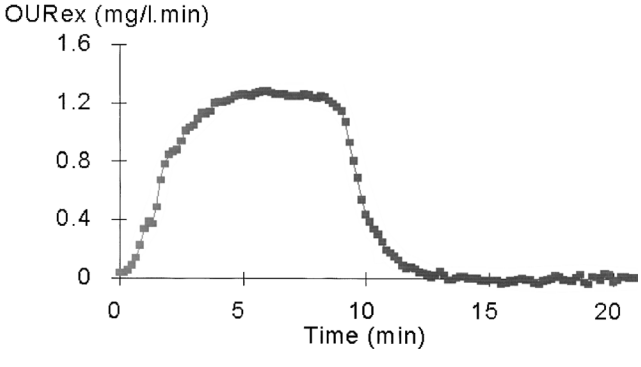

Figure 5. OUR profile (where endogenous OUR level was subtracted from the original OUR data) for a pulse addition of acetate to a *Pseudomonas aeruginosa* culture at $t = 0$.

Empirical Modeling of Transient Response of Activated Sludge in Batch Experiments

First-Order Model

The observed transient phenomenon can be modeled in a very simple but appropriate way by means of the following first-order model of the observed growth rate:

$$\mu_{obs} = Trans \cdot \mu \tag{3}$$

$$Trans = (1 - e^{-t/\tau}) \tag{4}$$

where *Trans* is the transient term associated with the first-order model (dimensionless), τ is the first-order time constant (T), t is time (T), μ_{obs} is the observed specific growth rate of the activated sludge (T^{-1}), and μ is the maximum specific growth rate of the activated sludge (T^{-1}).

This first-order approach was used by Vanrolleghem and co-workers as early as 1992 (De Schryver, 1992). Since then, the first-order model has been commonly mentioned and used by the same group in studies dealing with various applications of respirometric batch experiments (Gernaey et al., 2001, 2002a, 2002b; Sin et al., 2003; Vanrolleghem and Spanjers, 1998; Vanrolleghem et al., 1998). Quite recently, Guisasola et al. (2003) independently adopted the first-order model, but still without providing a plausible explanation of the observed transient phenomenon.

The concept of the empirical model is illustrated in Figure 6. The Monod-based model prediction of the OUR is

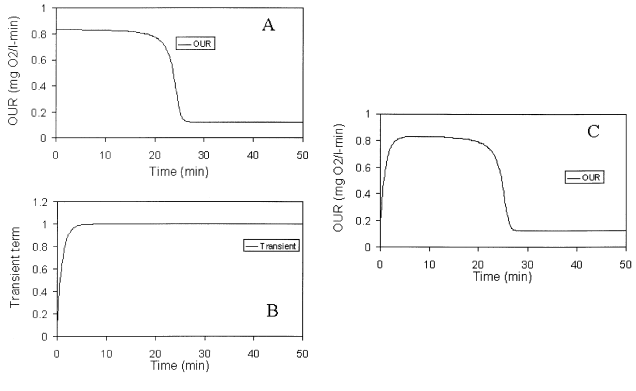

Figure 6. A typical simulated OUR profile in batch experiments using Monod kinetics (refer to Fig. 1 for simulation conditions) (A). The simulated first-order model transient for $\tau = 1$ min (B). Simulated OUR obtained by multiplying the OUR based on Monod kinetics with the first-order model, resembling the experimental observations (see Fig. 2) (C).

given in Figure 6A, whereas the first-order model of Eq. (3) was simulated for a time constant of 1 min (Fig. 6B). The transient response observed in experimental OUR data can be reproduced almost perfectly by multiplying the Monod model prediction with the first-order model. It is obvious that employing this rather simple empirical approach is only one of possible solutions for modeling batch experiments.

It should be noted that this approach implies that there is not only a transient in OUR but also in the substrate uptake. This is because, from a modeling point of view, the substrate and oxygen uptake are both directly linked to the growth process in ASM1-like models (Henze et al., 2000). This is addressed in what follows.

Application of First-Order Model to Experimental Data

The empirical first-order model of Eqs. (3) and (4) is applied to experimental data sets. The mathematical model that incorporates the first-order response model is presented in a compact matrix format in Table I (see Henze et al. [2000] for detailed information).

Batch Experiments With Aerobic Activated Sludge (Heterotrophs)

A typical response of an activated sludge culture to a pulse addition of carbon source (here acetate) is shown in Figure 7. The biomass OUR reaches its maximum level after a transient phenomenon. A discussion of the titrimetric data shown in this figure will follow. As shown in Figure 7, the first-order model describes the transient response occurring in the OUR profiles sufficiently well. In general, the first-order time constants observed with aerobic heterotrophs seemed to change with the different activated sludge samples (Table II).

Batch Experiments With Aerobic Nitrifiers

The transient phenomenon was also observed in batch experiments with ammonium pulse additions for the kinetic characterization of nitrifying biomass (Fig. 8). Similar to the heterotrophic bacteria, the OUR of the nitrifiers reaches a maximum OUR after a transient response. A prolonged transient is observed with nitrifiers subjected to 1-day famine (aerobic endogenous respiration) conditions as demonstrated with the open square-marked OUR in the same figure. This change in the time constant might be resulting from physiological adjustments of the nitrifiers in response to changing environmental conditions (i.e., enzyme activity level, protein synthesis level, etc.). This hypothesis is discussed in what follows.

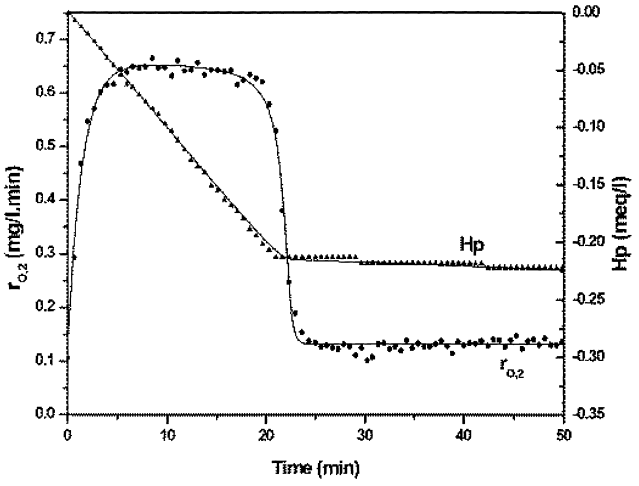

Figure 7. The response of aerobic heterotrophs to a pulse addition of (49.72 mg COD/L) acetate. Model fits to the OUR and Hp (titrimetric data) are also shown (Gernaey et al., 2002b).

Bias Induced in Biodegradation Parameters by Neglecting the Transient Phenomenon

The bias induced in parameters estimated from respirograms obtained by not properly accounting for the transient phenomenon is illustrated for the two experiments taken from Vanrolleghem et al. (1995) (Fig. 9), and for one experiment performed specifically for this study (see Table III).

Table II. Estimates of first order time constants and substrate affinity constants for various activated sludge activities (S_{NH} (0) and K_{NH} are relevant for experiments with nitrifiers; $S_S(0)$ and K_S are relevant for experiments with heterotrophs).

Exp. id.	Initial substrate $S_{NH}(0)$ or $S_S(0)$	Substrate affinity K_{NH} or K_S	τ (min)	Reference
Experiments with nitrifiers: ammonium as substrate source				
1	5.02	0.43	2.907	Gernaey et al. (2001)
Day 1	3.3	0.25	0.91	Spanjers and Vanrolleghem (1995)
Day 2	3.3	0.15	3.0	Spanjers and Vanrolleghem (1995)
Experiments with aerobic heterotrophs: acetate as substrate source				
1	25.22	0.72	1.88	Gernaey et al. (2002b)
2	37.61	0.63	1.74	Gernaey et al. (2002b)
3	49.72	0.62	1.42	Gernaey et al. (2002b)
4	61.39	0.58	1.7	Gernaey et al. (2002b)
5	45.9	0.66	1.25	This study
6	48.4	0.72	2.24	Kotte (2002)

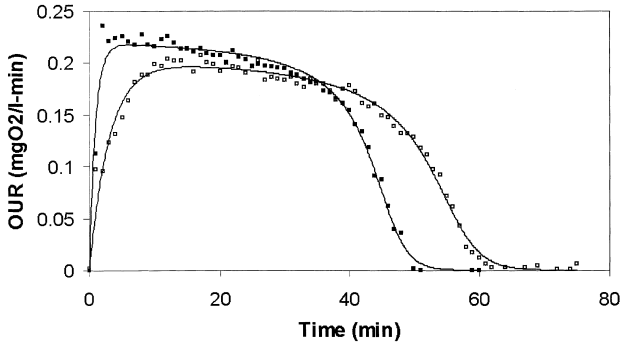

Figure 8. Transient response of nitrifying biomass to a pulse addition of 3.3 mg ammonium in a 1.4-L batch reactor. Filled markers indicate day 1; open markers indicate experiment at day 2; smooth lines indicate simulation results (Spanjers and Vanrolleghem, 1995).

The original experimental data of Vanrolleghem et al. (1995) shown in Figure 9A,C clearly show the transient phenomenon. To be able to model this using Monod kinetics, the investigators cut out the transient period from the original data and used only that part of the data that could be explained by the Monod model (Fig. 9B,D). The original complete data sets were used to compare the parameter estimation results with or without considering the transient period. When considered, the transient period is modeled using the first-order model introduced previously. For the parameter estimation (PE) procedure, it is assumed that no significant growth takes place during the short-term batch experiments and that the initial biomass concentration is fixed to 2000 mg COD/L. Moreover, the initial amount of substrate added is known a priori. Under these assumptions, the identifiable parameter combinations shown in Table III can be estimated using OUR as a measured variable (Dochain and Vanrolleghem, 2001).

The results (Table III, Fig. 9) show that ignoring the transient period leads to a considerable underestimation of the yield coefficient, Y_H. This is because the identifiable parameter combination, $(1 - Y_H)S_S(0)$, changes whether or not the transient period is considered (see Table III). Because the initial amount of substrate addition is known a priori, the error made in the estimate of $(1 - Y_H)S_S(0)$ propagates directly into the estimate of Y_H. This result is not surprising because modeling the transient period provides a more accurate calculation of the area under the OUR profile (see Fig. 9). This area is used basically to estimate the Y_H, as it determines the oxygen consumed for degradation of the substrate.

One could argue that Y_H could be estimated separately by calculating the area under the measured OUR curve (rather than by fitting a whole model to it). However, by working in this way a bias may be introduced in the other parameters (Dochain and Vanrolleghem, 2001). All parameters should be estimated simultaneously.

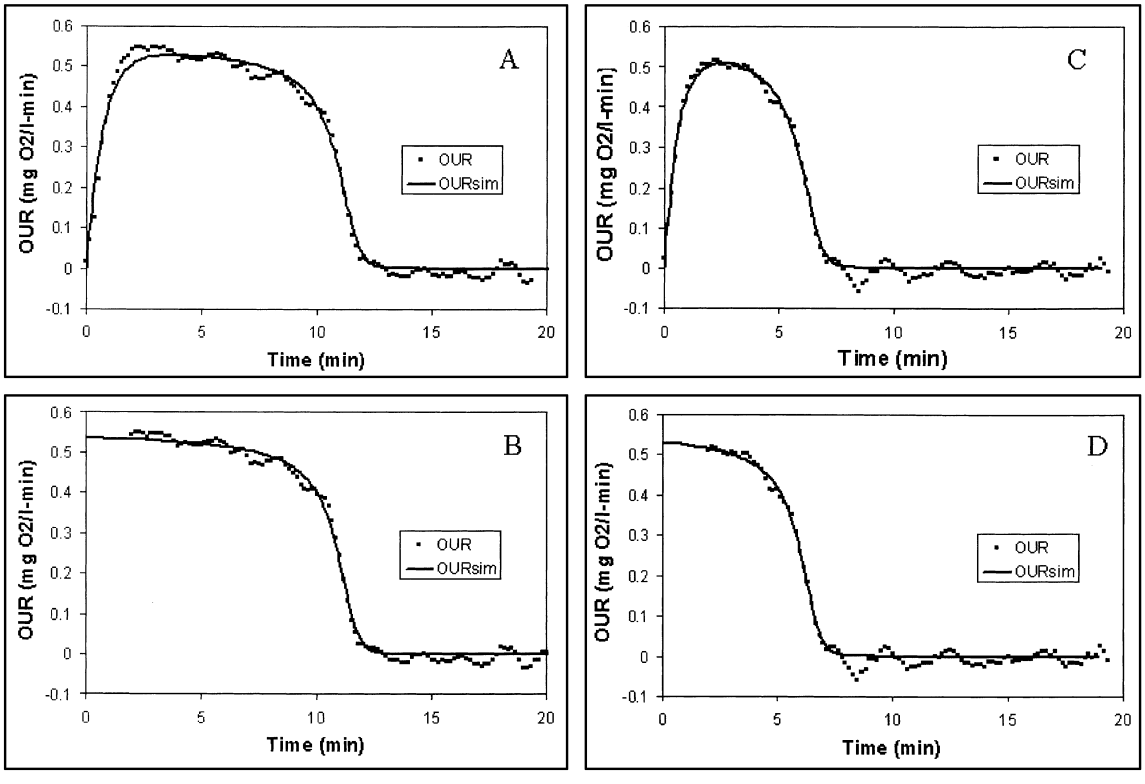

Figure 9. OUR profiles obtained from pulse addition of 20 mg COD/L acetate (A,B) and 10 mg COD/L (C,D) to an aerobic activated sludge sample. Model fits are based on an ASM1 model. Deleting the transient responses from the OUR data (B, D) is compared with first-order dynamic modeling of the transient phenomenon (A,C) (data from Vanrolleghem et al., 1995).

Table III. Parameter estimation results with and without considering the transient response.

PE approach	Duration[b]	$S_S(0)$	$\frac{1-Y_H}{Y_H}\mu_{\max H}X_H(0)$	$(1-Y_H)S_S(0)$	$(1-Y_H)K_S(0)$	Y_H	$\mu_{\max}H$	K_S	τ
Experiment A from Vanrolleghem et al. (1995)									
Model transient	12	20	0.57	5.2	0.18	0.74	0.00081	0.70	0.7
Ignore transient	12	20	0.57	5.6	0.17	0.72	0.00073	0.61	
ε_R[a] (%)						−2.78	−10.38	−14.75	
Experiment C from Vanrolleghem et al. (1995)									
Model transient	7	10	0.58	2.7	0.18	0.73	0.00078	0.67	0.61
Ignore transient	7	10	0.57	3.0	0.17	0.70	0.00066	0.55	
ε_R[a] (%)						−4.29	−18.18	−21.82	
Experiment from this study									
Model transient	30	45.9	0.40	10.2	0.15	0.78	0.00071	0.66	1.25
Ignore transient	30	45.9	0.40	10.6	0.13	0.77	0.00067	0.58	
ε_R[a] (%)						−1.30	−5.97	−13.79	

See text for details about the estimation procedure. Units for parameters given in the Nomenclature section.
[a]Relative error.
[b]Time needed to oxidize the initial amount of carbon source (i.e., duration of OUR due to external carbon source).

An error in the estimate of Y_H is quite critical because the estimate of Y_H is used to calculate $\mu_{\max H}$ and K_S from their respective parameter combinations (see Table III). The error in the estimate of Y_H is thus propagated into the estimate of $\mu_{\max H}$ and K_S. For instance, Y_H is underestimated by 2.78% in experiment A (see Table III). As a result, the estimates of $\mu_{\max H}$ and K_S are underestimated by 10.38% and 14.75%, respectively. It is significant to stress that the absolute value of the estimate of the identifiable parameter combination,

$$\frac{1-Y_H}{Y_H}\mu_{\max H}X_H(0)$$

does not change when ignoring the transient period (see Table III), because the error in Y_H is compensated by an error in $\mu_{\max H}$ as mentioned previously. Clearly, it can be concluded that ignoring the transient period in the original OUR data induces a bias in the separate parameter estimation results.

The absolute errors in the parameter estimation results depend on both the initial amount of substrate pulse and on the duration of the transient period (see Table III). Obviously, one could minimize the bias in the parameter estimates by increasing the amount of substrate injected. However, the initial amount of substrate addition in short-term batch experiments should be limited; that is, a low S_0/X_0 ratio should be applied, to prevent the biomass from significantly altering/adapting its physiological state during the experiment (Chudoba et al., 1992; Grady et al., 1996; Novák et al., 1994). Consequently, performing short-term batch experiments with small substrate additions is the usual practice when aiming at experimental data that should provide representative, so-called ''extant,'' kinetic information to be used later for the calibration of full-scale WWTP models. Having recognized the impact of the transient period on the parameter estimates from short-term batch experiments, it can be inferred that the transient phenomenon may be one of the reasons contributing to the variability in the kinetic parameter estimates in the literature (Grady et al., 1996).

DISCUSSION

In this general discussion, we answer the following question: To what extent is the transient response observed in the batch OUR profiles related to a biological response or, more particularly, to the substrate metabolism at the cellular level of the activated sludge culture? Glycolysis is used to illustrate the concept. The elucidation of the dynamics of cellular metabolism and its regulation is a challenging field of metabolic engineering (Stephanopoulos and Stafford, 2002) and results obtained in this field are used here to support the substrate metabolism hypothesis.

Fundamental Understanding

Carbon source degradation evaluated at the cellular level of heterotrophic bacteria is not a simple and straightforward process, but involves a complex system in which a myriad of enzymes, intermediate metabolites, and metabolic pathways are coordinated and regulated (Kramer and Sprenger, 1991). An illustrative and detailed account of glucosemetabolism was illustrated by Theobald et al. (1997) for the yeast *Saccharomyces cerevisiae*.

Theobald et al. (1997) measured in vivo dynamics of the metabolites produced from glucose metabolism by *S. cerevisiae* after pulse addition of glucose to a steady-state culture of *S. cerevisiae* (shown in the Fig. 7 of Theobald et al. [1997]).

A very dynamic change in the intracellular metabolite concentrations can be observed upon pulse glucose addition.The time constant of the dynamic response in the intracellular metabolites to this pulse varies as the carbon (glucose) flux moves downward into the central metabolic pathway shown in Figure 1 of Theobald et al. (1997). For

example, the time constant of the dynamic response observed in the glucose-6-phosphate (G6P) concentration is around 5 s, whereas the time constant of the dynamic response observed in the glyceraldehydes-3-phosphate (GAP) concentration, which is situated in the middle of the glycolysis pathway, is already around 30 s. It is important to note here that the respiration rate measurements (OUR) take place at the end of the catabolic pathway. That means that the time constant of the respiration response would be expected to be even higher.

Quite recently, in a dynamic metabolic modeling study of *Escherichia coli* (Chassagnole et al., 2002), it was shown that the time constants of the numerous steps involved in the metabolic network of the central carbon metabolism of *E. coli* vary between 29 ms and 85 s. It can be inferred from the time constants identified for central carbon metabolism that delays in the substrate metabolism might be the underlying mechanism of the transient phenomenon observed in the OUR measurements.

The substrate metabolism hypothesis is demonstrated schematically in Figure 10. Pulse addition of the substrate is the input to the system and the OUR measurements can be considered the measurements of the output (i.e., the pulse response of the system). In this case, the resulting transient phenomenon observed in the OUR measurements is dependent on the characteristic time of the system, which is the entire metabolic network of the cell. The left-hand side of Figure 10 illustrates the substrate metabolism and its regulation to fine-tune the substrate flux through the different metabolic pathways, which is determined essentially by the physiological state of the cell. For example, if the cell is exposed to feast and famine conditions then the cell will probably optimize the storage and growth processes to gain a selective advantage in famine conditions (Daigger and Grady, 1982; van Loosdrecht and Heijnen, 2002).

Input/Output Behavior: Substrate Uptake Rate/OUR Measurements in Batch Experiments With Aerobic Carbon Source Degradation

In aerobic batch experiments with acetate, the substrate uptake rate can be monitored indirectly using the titrimetric methodology of Gernaey et al. (2002a). This titrimetric methodology for substrate uptake monitoring is based on the fact that, at pH 7 to 8, the acetate is present in dissociated form. Consequently, because it is the undissociated form that is taken up, every millimole of acetate consumed removes approximately 1 mmol of protons from the medium. This

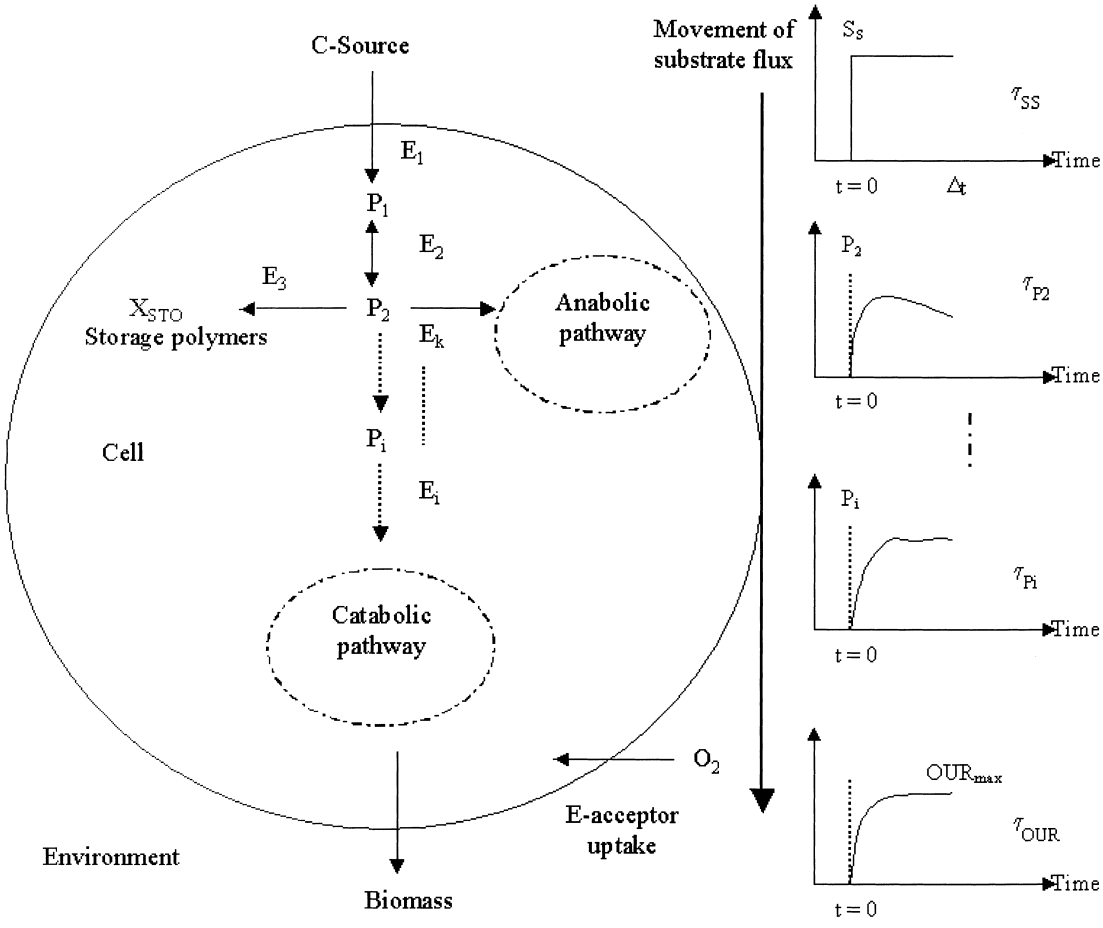

Figure 10. An illustrative description of the substrate metabolism hypothesis for the activated sludge mixed culture.

proton consumption is compensated by acid addition in the experimental set-up, and this addition is recorded during the experiment. For a detailed explanation of this titrimetric methodology, the reader is referred to Gernaey et al. (2002a).

Table IV summarizes the range of time constants for the transient phenomenon observed in OUR and Hp measurements, and the corresponding references to the publications with the experimental data. In general, it is observed in aerobic experiments that the acetate uptake after a pulse addition is a very fast process with hardly any detectable transient phenomenon (see Fig. 7)—that is, with a very small time constant (0.12-min average in Table IV). The OUR data result from a slower process and therefore exhibit a clear initial transient phenomenon, characterized by a longer time constant (1.74-min average in Table IV).

A result of the difference between titrimetric and OUR data in experiments with acetate are the differences in estimated biomass Monod substrate affinity constants. The sharp bending point at the end of the substrate degradation process observed in the Hp data results in very low substrate affinity constants being estimated from Hp data (Fig. 7). The average K_S estimate was 0.25 mg COD/L. For comparison, the OUR data resulted in an average K_S estimate of 0.68 mg COD/L. These results mean that the substrate uptake process suddenly stops (according to high affinity-transport enzyme kinetics) when the substrate is exhausted from the medium, whereas the OUR only slows down at a slower pace after substrate is depleted due to the intracellularly accumulated intermediates that can still be oxidized.

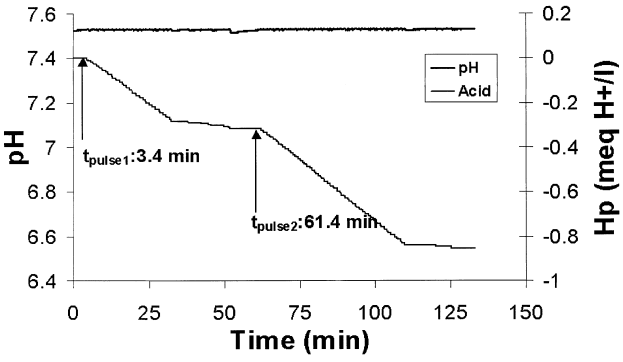

Figure 11. Consecutive addition of two acetate pulses to an anoxic batch reactor. The first acetate pulse (23.5 mg COD/L) was given to the reactor at 3.4 min, SNO3(0) = 12.5 mg N/L. The second acetate pulse (46.7 mg COD/L) was injected to the anoxic reactor at 61.4 min, SNO3(0) = 8.6 mg N/L.

Input/Output Behavior: Batch Experiments With Anoxic Acetate Degradation

Anoxic respiration of activated sludge as measured by the titrimetric method proposed elsewhere (Petersen et al., 2002; Sin et al., 2003) is shown in Figure 11. The titrimetric data in this experiment are assumed to be the combined effect of four processes influencing the proton concentration of the medium. These processes, explained in detail by Petersen et al. (2002) and Sin et al. (2003), are: substrate (carbon source) uptake (proton consumption effect); nitrate uptake as electron acceptor (proton consumption effect); CO_2 production due to biomass respiration (proton production effect); and ammonia uptake for growth (proton production effect). Anoxic oxidation of acetate typically results in acid addition for pH control during titrimetric experiments, indicating a consumption of protons from the medium. The analysis by Petersen et al. (2002) shows that the proton consumption is basically due to rapid uptake of acetate as carbon source and nitrate uptake as electron acceptor. Once the acetate is removed from the medium, which took about 28 min in this experiment, the acid addition decreases drastically to a background rate determined by the pH effects of endogenous processes. For the first pulse addition of acetate (23.5 mg COD/L), acid addition starts early to keep the pH of the medium constant, following a transient period with a time constant of 0.47 min (see Fig. 11). For the second pulse addition of acetate (46.7 mg COD/L), the transient response phenomenon is repeated with a time constant of 0.42 min.

The time constants observed on titrimetric data with anoxic acetate oxidation are three times (on average) higher than the values for aerobic acetate oxidation. This difference might be caused by the difference in the metabolism of acetate under aerobic and anoxic conditions. More probably, the titrimetric data under anoxic conditions are also influenced by nitrate uptake, which is not the case for the aerobic conditions where oxygen uptake has no pH effect on

Table IV. Estimates of first order time constants and substrate affinity constants for aerobic activated sludge in various experiments with acetate addition as carbon source.

Exp. no.	$S_S(0)$ (mg COD/L)	τ_{HP} (min)	τ_{OUR} (min)	K_{S_HP} (mg COD/L)	K_{S_OUR} (mg COD/L)	Reference
1	25.22	0.18	1.88	0.3	0.72	Gernaey et al. (2002b)
2	37.61	0.1	1.74	0.1	0.63	Gernaey et al. (2002b)
3	49.72	0.1	1.42	0.1	0.62	Gernaey et al. (2002b)
4	61.39	0.1	1.7	0.6	0.58	Gernaey et al. (2002b)
5	72.49	0.13	1.95	0.17	0.71	Gernaey et al. (2002b)
6	45.9	—	1.25	—	0.66	This study
7	48.4	—	2.24	—	0.72	Kotte (2002)
Average		0.12	1.74	0.25	0.68	

the titrimetric data. As a result, the titrimetric data under anoxic conditions will reflect lumped dynamics of two processes: (1) acetate uptake determined by the very fast substrate uptake kinetics; and (2) nitrate (electron-acceptor) uptake driven mainly by the growth kinetics.

In summary, the experimental observations with (aerobic and anoxic) activated sludge activities provide additional support for the aforementioned substrate metabolism hypothesis. Regarding aerobic acetate oxidation, the time constant of the substrate uptake process, which is monitored by the titrimetric data, is on the order of 0.1 min, with a very high substrate affinity (K_S = 0.25 mg COD/L), indicating that substrate uptake is a rapid process with fast dynamics. On the other hand, the time constant obtained from the OUR data is on the order of 1.75 min, with a relatively lower estimated substrate affinity (K_S = 0.68 mg COD/L), indicating that the OUR has comparatively slower dynamics. In their experimental studies, van Loosdrecht and Heijnen (2002) observed that the substrate uptake process is a very fast process compared with the growth process.

These experimental data fit very well with the hypothesis formulated in Figure 10. The substrate uptake as a starting point in the metabolic network of the cell has fast dynamics and does not depend on preceding reactions (Chassagnole et al., 2002). Moving down the chain of metabolic reactions, some time passes until the electrons of the substrate finally reach the oxygen (or nitrate) reduction sites situated at the end of the metabolic network. As a result, a dynamic transient response occurs in the uptake of oxygen from the surrounding environment, which can be observed from the OUR data. It can be expected that the dynamics determining the transient phenomenon will depend on the structure of the metabolic network, which is also determined by the physiological state of the culture. In this regard, this might explain the change in the time constant of the nitrifiers when having a different culture history (see Fig. 8 and Table II).

It is obvious that modeling the transient phenomenon occurring in batch experiments should be possible with mechanistic dynamic metabolic network models (e.g., Chassagnole et al., 2002). However, such an approach is an extremely complex solution, which in the case of activated sludge mixed cultures is hardly applicable. However, the formulated hypothesis helps to understand the observed phenomenon from a mechanistic point of view.

The approach adopted here—that is, a simple empirical first-order process—works well to describe the batch experimental results and allows one to obtain good biokinetic parameters despite the transient phenomenon. Moreover, it allows for the study of differences in observed time constants (e.g., difference in the time constants of the substrate uptake versus oxygen uptake; effect of famine state on the time constants for the nitrifiers), which led us to the metabolic hypothesis just presented.

If we want to adopt this first-order approach to situations other than those encountered in a batch experiment, a mathematical formulation other than the one in Eqs. (3) and (4) will be required. A very natural approach would be to introduce an additional state variable; for example, the intracellular substrate that is linked to the external substrate via a first-order differential equation. This would also allow for decoupling of substrate uptake from the growth process. However, when such approach is adopted, considerable problems surface when confronting substrate uptake data and oxygen uptake data for complete batch experiments (results not shown). Further research is currently ongoing to find a simple mathematical description of the observations that would also work under fast-alternating conditions other than batch experiments (e.g., the example given in Fig. 12, where substrate addition took place both at t = 0 min and t = 11.6 min).

Significance of Modeling Transient Phenomenon for Activated Sludge Processes

One may wonder whether the observed discrepancy between model and experimental data has any practical importance. First, explicitly accounting for the transient phenomenon in the data interpretation is important to achieve correct interpretation of the experimental data; that is, it will result in more realistic model parameter estimates, as illustrated in Table III. It was shown that if the transient period is not taken into account, erroneous biomass yield values would result from short-term batch experiments. Moreover, an error in the yield coefficient is amplified into the estimates of the maximum specific growth rate and the substrate affinity constant, with error rates as high as 18% and 22%, respectively (see Table III).

When approximating the observed transient phenomenon by a first-order model, the time constant of the transient phenomenon is typically on the order of minutes. Thus, such transient phenomena cannot be ignored for many types of full-scale WWTPs, where the biomass is exposed to fast alterations in substrate and electron-acceptor concentrations. In this respect, the similarities between batch substrate degradation experiments and full-scale plug-flow WWTP

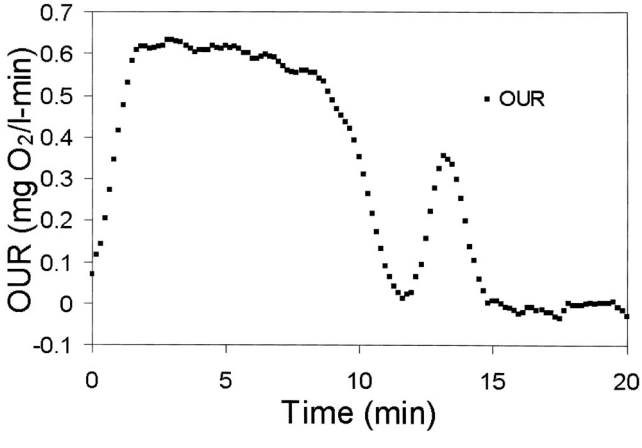

Figure 12. Repeatability of the transient phenomenon in response to pulse additions of acetate during a batch degradation experiment. The first pulse is 20 mg COD/L, and the second pulse is 4 mg COD/L (Dochain and Vanrolleghem, 2001).

behavior should be considered. In both systems, rapid biomass activity changes occur. In the batch experiment, biomass activity will quickly change after batch substrate addition (see Fig. 2). Once all substrate is consumed, the measured OUR quickly decreases to zero, and the biomass resumes its endogenous state. In many full-scale plug-flow-like activated sludge systems, such as oxidation ditches (Metcalf and Eddy, 1991) or Orbal WWTPs (Daigger and Littleton, 2000), the activated sludge is cycled around a circular reactor, thereby passing the influent feeding point every 5 to 15 min, depending on configuration and operating parameters. During each cycle, the biomass can undergo aerobic, anoxic, and anaerobic conditions. Thus, in these full-scale systems, the activated sludge is subject to batch-like conditions similar to pulse substrate additions every time it passes the influent feeding point.

Rapidly changing conditions similar to those induced by a pulse substrate addition also take place in fast-alternating systems; for example:

(a) They occur in sequencing batch reactors (SBRs) (Demuynck et al., 1994) or standard activated sludge treatment plants (Wouters-Wasiak et al., 1994) with high-frequency intermittent aeration cycles or alternating aeration plants, such as Biodenitro/Biodenipho systems (Bundgaard et al., 1989).
(b) They occur in plug-flow-type pre-denitrification systems (Wentzel et al., 1992) with short anoxic residence times, particularly when considering the single-pass hydraulic retention time in the reactor; that is, the ratio of the volume of the reactor to the total inflow to the reactor (influent plus recycle plus internal recirculation flows).
(c) Plug-flow-type selectors, usually situated in front of the main aeration tanks (plug-flow and/or carousel) to enhance biological phosphorus removal and prevent sludge bulking, have a short hydraulic retention time (e.g., in the order of 10 to 25 min as reported in Meijer et al., 2002), which implies even shorter single-pass biomass residence times on the order of minutes (Vanrolleghem et al., 2003).
(d) They occur in oxidation ditch systems with short phases, such as Orbal plants (Daigger and Littleton, 2000) and carousel plants (Meijer et al., 2002; Vanrolleghem et al., 2003), where circulation times are also on the order of minutes.

In many types of biological WWTPs, the transient phenomenon can therefore be expected to occur and it will be an inherent characteristic of the biomass activity influencing the process every time the biomass is exposed to feast (feed) conditions. This is further illustrated in Figure 12, where substrate addition took place both at t = 0 min and t = 11.6 min. In both cases the transient phenomenon is clearly observed. Therefore, proper understanding and modeling of the transient phenomenon can contribute to improved full-scale WWTP modeling. Inclusion of the transient behavior in full-scale models requires extension of the first-order model [Eq. (3)], which is applicable only to batch reactors to continuous systems. Preliminary investigations (not shown) have indicated that development of such a continuous model is not straightforward, and it is the subject of ongoing research. It is important to mention already, however, is that it requires a description of at least three processes—that is, substrate uptake, metabolism in the metabolic network (as a first-order [or higher] system) and growth—instead of just the single process (i.e., growth) of ASM1 (Henze et al., 2000).

CONCLUSIONS

The underlying mechanisms of the transient phenomenon often observed in the experimental data (e.g., OUR) obtained from short-term batch assays with activated sludge following a pulse substrate addition was investigated in detail in this study. The detailed analysis of the dynamics of the experimental set-up only partially explains the observed transient phenomenon. Among the hypotheses proposed it appears that the transient response of activated sludge after a pulse substrate addition most likely results from the metabolism of the substrate.

It was shown that the transient phenomenon can be described by a first-order model. It was demonstrated that an erroneous estimate of the biomass yield is obtained if the transient period is not taken into account during model-based parameter estimation. This error is propagated (and amplified) into the estimates of the maximum specific growth rate and the substrate affinity constant.

The first-order time constant of the transient phenomenon observed in the experimental data shows that the substrate uptake process has a faster response (0.12 min, on average) compared with the oxygen uptake dynamics (1.74 min, on average), which basically reflects the dynamics of the overall substrate degradation process in the model. These results suggest that the substrate uptake processes should be decoupled from the growth processes in future activated sludge WWTP models.

By properly taking this transient phenomenon into account we can expect to yield better understanding and modeling of fast-alternating biological systems such as Orbal, carousel-type treatment, or other fast-alternating plants, but a complete analysis of its impact is beyond the scope of the present study.

NOMENCLATURE

ASM	activated sludge model
ASM1	activated sludge model number 1
AUR	ammonium uptake rate (mg NH_4-N/L·min)
COD	chemical oxygen demand (mg COD/L)
DO	dissolved oxygen (mg O_2/L)
E	output of the DO electrode (mg O_2/L)
Hp	proton concentration in mixed liquor (mEq/L)
HpR	proton production rate (mEq/L·min)

i_{XB}	nitrogen content of biomass (mg N/mg COD)
K_{NH}	affinity coefficient of autotrophs for ammonium nitrogen (mg NH_4-N/L)
K_S	heterotrophic substrate affinity coefficient (mg COD/L)
K_{S_HP}	K_S estimate based on Hp data (mg COD/L)
K_{S_OUR}	K_S estimate based on OUR data (mg COD/L)
m	degree of mixing
NH_4^+-N	ammonium nitrogen (mg NH_4-N/L)
NUR	nitrate uptake rate (mg NO_3-N/L·min)
OUR	oxygen uptake rate (mg O_2/L·min)
r_{O_2}	oxygen uptake rate (mg O_2/L·min)
$S_{NH}(0)$	initial ammonium concentration (mg N/L)
S_{NH}	ammonium concentration (mg N/L)
$S_{NO_3}(0)$	initial nitrate concentration (mg N/L)
SRT	sludge retention time
$S_S(0)$	initial readily biodegradable substrate concentration (mg COD/L)
S_O	actual DO concentration in the reactor (mg O_2/L)
S_S	readily biodegradable substrate concentration (mg COD/L)
$s(t)$	concentration of tracer at time t
s_0	initial concentration of tracer
s_∞	concentration of tracer as $t \rightarrow \infty$
S_0/X_0	initial substrate to biomass ratio (mg COD/mg COD)
t_{pulse}	time of pulse addition of substrate (min)
t_m	mixing time (min)
Trans	first-order dynamic transient term (-)
WWTP	wastewater treatment plant
Y_A	autotrophic yield coefficient (mg COD/mg NH_4-N)
Y_H	heterotrophic yield coefficient (mg COD/mg COD)
X_A	autotrophic biomass concentration (mg COD/L)
X_H	heterotrophic biomass concentration (mg COD/L)
ε_R	relative error (%)
μ	specific growth rate for heterotrophic biomass (min^{-1})
μ_{maxH}	maximum specific growth rate for heterotrophic biomass (min^{-1})
μ_{maxA}	maximum specific growth rate for autotrophic biomass (min^{-1})
μ_{obs}	observed specific growth rate for heterotrophic biomass (min^{-1})
τ	first-order time constant (min)
τ_A	first-order time constant observed in autotrophic activity (min)
τ_H	first-order time constant observed in heterotrophic activity (min)
τ_{HP}	first-order time constant estimated based on Hp data (min)
τ_{OUR}	first-order time constant estimated based on OUR data (min)

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