Respirometric assessment of storage yield for different substrates

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Abstract A new procedure has been defined for the respirometric assessment of bacterial storage yield as defined in the Activated Sludge Model No.3. The procedure was used to determine the storage yield, Y_{STO}, associated with *acetate*, *glucose* and *domestic sewage*, together with mixtures of *acetate*/*glucose* and *acetate*/*domestic sewage* at different initial F/M ratios. Y_{STO} was calculated as 0.78 gCOD(gCOD)$^{-1}$ for *acetate*, 0.87 gCOD(gCOD)$^{-1}$ for *glucose* and 0.96 gCOD(gCOD)$^{-1}$ for *domestic sewage*. The Y_{STO} of substrate mixtures was found to reflect the characteristics of the dominant fraction in the mixture.

Keywords Acetate; Activated Sludge Model No.3; domestic sewage; glucose; oxygen utilisation rate; respirometry; storage yield

Introduction

Studies conducted on both pure and mixed activated sludge cultures provided substantial proof on the ability of microorganisms to convert substrate into internal storage products under dynamic conditions (van Loosdrecht *et al*., 1997). The growth response of heterotrophic microorganisms was observed to take place mainly at the expense of stored products (Majone *et al*., 1999). Substrate storage was also recognised as a key process for biological phosphorus removal (Wentzel *et al*., 1986; Mino *et al*., 1987). It was recently introduced within *activated sludge model no.3* (ASM3), a comprehensive new modelling approach for activated sludge cultures, as the first step for the utilisation of readily biodegradable substrate (Gujer *et al*., 2000). The stoichiometry and kinetics of biological storage have been defined in ASM3, but the experimental procedures for the determination of the coefficients associated with the process kinetics have not been standardised yet.

The storage yield Y_{STO}, is one of the most important parameters of the model, since it represents the stoichiometric amount of the substrate converted into storage products, which are then utilised for growth. The assessment of storage yield is therefore crucial for the accurate estimation of the overall electron acceptor utilisation and sludge production. Although certain complicated techniques were suggested for its assessment (Goel *et al*., 1998; Dircks *et al*., 1999), very little experimental data of practical value for activated sludge systems is so far available.

This paper presents an experimental procedure, with conceptual justification, for the determination of the storage yield as defined in ASM3. It is based on respirometry, without involving measurement of storage products, as it will not always be possible or reliable to determine the amount of all the storage products when a complex substrate such as *domestic sewage* is concerned. The proposed procedure was tested on *acetate*, *glucose* and *domestic sewage*, together with mixtures of *acetate*/*glucose* and *acetate*/*domestic sewage*. Relevant experimental data were generated at different initial F/M ratios, enabling the identification of Y_{STO} values related to tested substrates and substrate mixtures.

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Proposed procedure for the calculation of Y_{sto}

The major feature of ASM3 differentiating it from other activated sludge models is the storage process, stipulating conversion of all biodegradable COD, either directly or through preliminary hydrolysis, into internal storage polymers. Growth is only allowed to occur at the expense of storage products. Storage is defined as a faster process compared to growth and may be identified as the dominant mechanism in a batch reactor. The reaction kinetics and stoichiometry of ASM3 for organic carbon removal, simplified for soluble biodegradable COD as the sole substrate component, as evaluated in this study, is given in Table 1. The basic stoichiometry of the storage process can be expressed as follows:

$$\Delta O_{STO} = (1 - Y_{STO})\,\Delta S_S \tag{1}$$

At the depletion of all initially available readily biodegradable COD, (S_{S1}), the above expression can be manipulated to give the storage yield, Y_{STO}:

$$Y_{STO} = \left(1 - \frac{\Delta O_{STO}}{S_{S1}}\right) \tag{2}$$

The above expression indicates the oxygen utilised for storage as a convenient parameter for the calculation of the storage yield. In fact, Y_{STO} would be computed if the oxygen used for the storage of a known amount of readily biodegradable COD could be determined by means of respirometric measurements. Such measurements however only provide the oxygen utilisation rate, (OUR), of the overall system and not the OUR specific for the desired process alone. Thus it is necessary to understand and interpret the components of an overall OUR associated with the biodegradation of a soluble substrate. A batch reactor is a perfect tool for this purpose as it exhibits the entire transient OUR responses.

In this context, the basis of the procedure was established with the theoretical interpretation of the oxygen utilisation rate in an aerated batch reactor. Figure 1 illustrates a typical OUR curve obtained through model simulation using AQUASIM® (Reichert *et al.*, 1998), for the kinetic and stoichiometric coefficients as suggested in ASM3 (Table 2). The simulation was performed for an F/M ratio of 1.0 gCOD(gcellCOD)$^{-1}$ and an initial active heterotrophic biomass concentration, X_H, of 200 mg/l COD. It was started without any initial substrate addition in order to determine the endogenous OUR level and feeding was initiated at the desired F/M ratio after this famine period.

The OUR profile obtained by model simulation can be fractionated, as shown in Figure 2(a), for each separate process incorporated in ASM3. The area under each curve gives the amount of oxygen used for a specific process. Thus, it is possible to calculate the area under

Table 1 Matrix representation of ASM3 simplified for soluble organic carbon

Component process	S_O O_2	S_S COD	X_I COD	X_H COD	X_{STO} COD	*Rate*
Storage of S_S	$-(1-Y_{STO})$	−1			Y_{STO}	$k_{STO}\frac{S_O}{K_O+S_O}\frac{S_S}{K_S+S_S}X_H$
Growth on X_{STO}	$-\frac{(1-Y_H)}{Y_H}$			1	$-1/Y_H$	$\mu_H\frac{S_O}{K_O+S_O}\frac{S_{NH}}{K_{NH}+S_{NH}}\frac{S_{HCO}}{K_{HCO}+S_{HCO}}\frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H}X_H$
Endogenous respiration	$-(1-f_I)$		f_I	−1		$b_H\frac{S_O}{K_O+S_O}X_H$
Respiration of X_{STO}	−1				−1	$b_{STO}\frac{S_O}{K_O+S_O}X_{STO}$

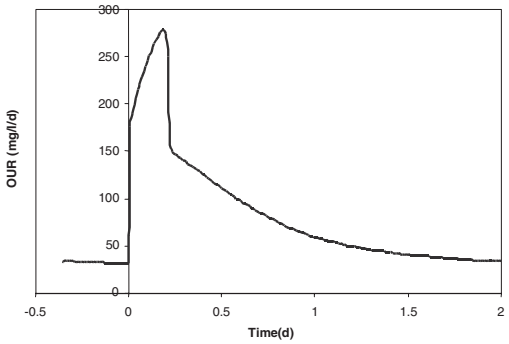

Figure 1 The OUR curve for F/M = 1.0 gCOD(gcellCOD)$^{-1}$

Table 2 Kinetic and stoichiometric coefficients used in model simulation

Model coefficient	Default value
k_{STO}	5 d^{-1}
K_S	2 mgl^{-1} COD
Y_{STO}	0.85 COD/COD
μ_H	2 d^{-1}
K_{STO}	1
Y_H	0.63 COD/COD
B_{STO}	0.2 d^{-1}
B_H	0.2 d^{-1}
f_I	0.2

the OUR curve associated with storage and the amount of oxygen utilised for the storage of all the readily biodegradable COD (ΔO_{STO}) by simple integration. It is also possible, through model simulation to subtract the OUR values of all three processes except that of storage from the overall OUR profile, to get the graphical magnitude of the area representing storage, as indicated in Figure 2(b).

This evaluation forms the basic structure of the proposed procedure for the graphical determination of the amount of ΔO_{STO} without model simulation. The procedure involves, as shown in Figure 3, drawing a line connecting the initial OUR level due to endogenous decay (the initial/average OUR_{EndDec} level), up to the inflection point of the overall OUR curve. This reflects the depletion of the initially available readily biodegradable COD and therefore, the end point of storage of the substrate. The sudden drop observed in the OUR value is incorporated with the completion of external substrate utilization including storage. The area above the drawn line directly yields oxygen used for storage, ΔO_{STO}.

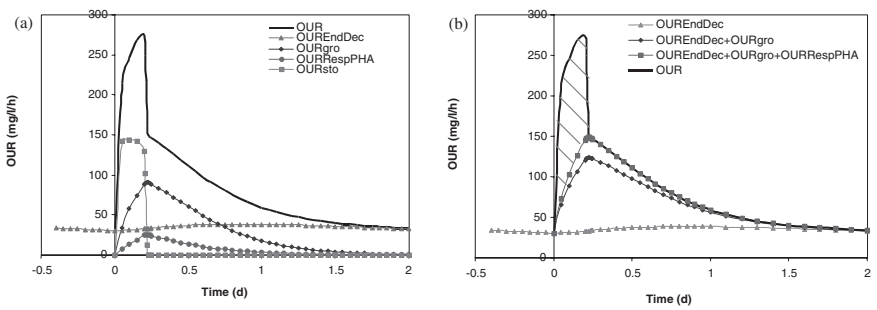

Figure 2 (a) Components of the OUR curve (b) Cumulative OUR curves for the identification of ΔO_{STO}

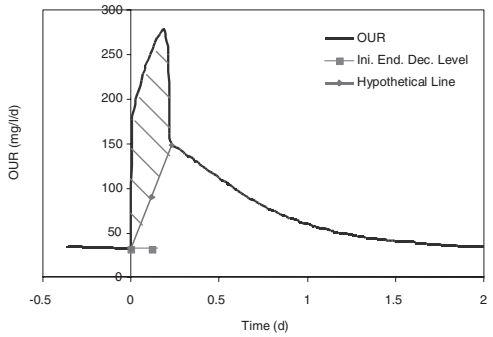

Figure 3 Proposed graphical procedure for the determination of ΔO_{STO}

The proposed procedure involves an approximation due to linear connection of two points under the OUR profile. A sensitivity evaluation was made for this purpose comparing theoretical model estimations with the calculated Y_{STO} values based on the proposed method for a wide range of F/M ratios between 0.02 and 1.5 gCOD(gcellCOD)$^{-1}$. Except for very low F/M ratios, corresponding to unrealistically low soluble biodegradable COD levels, the results indicate that the proposed procedure allows for the experimental assessment of Y_{STO}, with less than 2% error, excluding analytical errors associated with COD measurements (Karahan-Gül *et al.*, 2001).

Materials and methods

Respirometric measurements were conducted for the experimental testing of the proposed procedure on different substrates. *Acetate*, *glucose* and *domestic sewage* together with combinations of *glucose/acetate* and *domestic sewage/acetate* at different ratios were used as substrate. For single substrates, parallel tests were performed to visualise the effect of the initial F/M ratio on the magnitude of the storage yield. The tests were carried out in completely mixed, aerated 2–3 l batch reactors, seeded with active biomass taken from fill and draw acclimation reactors operated at steady state. The reactors were initially operated with no substrate for the assessment of the OUR level associated with the endogenous phase. The initial active biomass concentration was estimated by model simulation on the basis of an endogenous decay rate, b_H of 0.2 d^{-1}, the suggested value in ASM3. OUR measurements were conducted with a Manotherm RA-1000 continuous respirometer with PC connection. In the experiments, pH was kept in the range of 7.0–8.0, suitable for biological activity. COD measurements were performed as described in the method ISO6060 (1986). *Domestic sewage* used in the experiments was filtered through 0.45 μm cellulose acetate filters.

Experimental results

In the study, *acetate* was selected as the main compound for the testing of the proposed procedure. It is a well studied readily biodegradable substrate, known to be stored as polyhydroxybutyrate, (PHB), and recognised in biological phosphorus models as a significant model component. *Glucose* is also tested for the assessment of the corresponding storage yield, as it is known to trigger glycogen storage trough a metabolic pathway completely different from that of PHB storage. It is commonly agreed that the majority of the readily biodegradable substrate mixture in wastewaters is likely to be stored as PHA and glycogen. Therefore, these two pure substrates were selected as the precursors of the two extreme cases for the evaluation of the storage yield. *Filtered domestic sewage* was also used in the OUR experiments to reflect a real case example of a more complex readily biodegradable source for the proposed experimental procedure.

Experiments on acetate and glucose

Acetate experiments. Experiments on *acetate* were conducted as eight parallel runs for different initial F/M ratios varying in the range of 0.09–3.65 gCOD(gcellCOD)$^{-1}$. Y_{STO} values obtained by means of the proposed procedure, together with related experimental data, are outlined in Table 3. The resulting average Y_{STO} could be calculated as 0.78 gCOD(gCOD)$^{-1}$, slightly lower than the suggested average value of 0.85 gCOD(gCOD)$^{-1}$ in ASM3. This value is in agreement with the ones reported in the literature as 0.73 gCOD(gCOD)$^{-1}$ (van Aalst-van Leeuwen *et al*., 1997) and 0.69 (Beun *et al*., 2000). Y_{STO} was observed to change only within the narrow range of 0.75–0.82 gCOD(gCOD)$^{-1}$, leading to conclude that the F/M ratio did not have an appreciable effect on the magnitude of Y_{STO}. The F/M ratio only affected the shape of the OUR curve and the resulting ΔO_{STO}, as illustrated in Figure 4: an F/M ratio of 0.09 gCOD(gcellCOD)$^{-1}$, started with $S_{S1} = 93$ mgl^{-1} COD, generated only $\Delta O_{STO} = 23.3$ mgl^{-1} and yielded a Y_{STO} of 0.75 gCOD(gCOD)$^{-1}$. ΔO_{STO} was increased to 110.2 mgl^{-1} for F/M = 0.87 gCOD(gcellCOD)$^{-1}$ and S_{S1} = 548 mgl^{-1}, resulting in a slightly higher Y_{STO} of 0.80 gCOD(gCOD)$^{-1}$.

The proposed procedure calculates Y_{STO} in accordance with ASM3, with the assumption that available readily biodegradable substrate is entirely converted into internal storage products. There is experimental evidence however for partial storage allowing for simultaneous growth both competing for the same pool of external substrate (Majone *et al*., 1996; Beun *et al*., 2000; Dircks *et al*., 2001). It could then be argued that limiting external substrate at low F/M ratios would allow simultaneous microbial growth, as in conventionally operated activated sludge systems, whereas a sudden increase of external substrate with high F/M ratios after a famine period would highlight internal storage as the dominant mechanism. Since growth and storage are commonly defined by significantly different yield coefficients, the resulting weighted average yield value reflected by overall OUR measurements would exhibit a changing pattern parallel to adopted F/M values. The experimental results in this study provided a reliable indication that this was not the case for *acetate* utilisation, which was best interpreted with total storage.

Glucose experiments. Two runs were conducted on *glucose* with initial F/M ratios of 0.05 and 0.78 gCOD(gcellCOD)$^{-1}$. As indicated in Table 4, they both yielded a Y_{STO} of 0.87 gCOD(gCOD)$^{-1}$, a value significantly higher than for *acetate*. This value agrees well with a Y_{STO} of 0.9 mgCOD(mgCOD)$^{-1}$ assumed by Goel *et al*. (1999) and with the experimental results of Dircks *et al*. (2001) for *glucose*, with consideration that the formation of glycogen from *glucose* requires less energy as compared to PHB accumulation from *acetate*. It is stated that the maximum yield of glycogen from *glucose* is 46% greater

Table 3 Experimental assessment of Y_{STO} for *acetate*

Set No.	Reactor volume (l)	Biomass concentration (mg COD/l)	Substrate concentration (mg COD/l)	F/M ratio (COD/COD)	Y_{STO}
Set 1	2	1,000	93	0.09	0.75
Set 2	2	880	105	0.12	0.76
Set 3	2	1,450	280	0.19	0.76
Set 4	2	870	274	0.31	0.80
Set 5	2	440	187	0.42	0.75
Set 6	2	230	164	0.71	0.77
Set 7	2	630	548	0.87	0.80
Set 8	2	300	1,094	3.65	0.82
Average					***0.78***

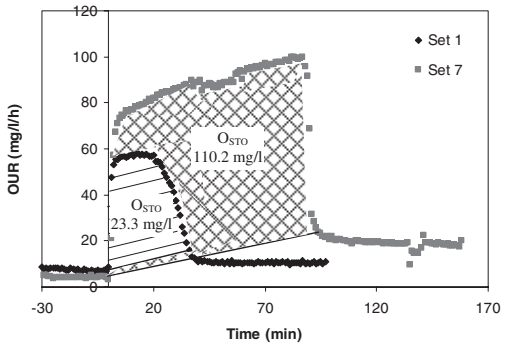

Figure 4 OUR profiles for *acetate*

Table 4 Experimental assessment of Y_{STO} for *glucose* and *glucose/acetate* mixtures

Set No.	Reactor volume (l)	Composition	Biomass concentration (mg COD/l)	Substrate concentration (mg COD/l)	F/M ratio (COD/COD)	Y_{STO}
Set 1	2.5	*Glucose*	260	200	0.78	0.87
Set 2	2.5	*Glucose*	1,000	50	0.05	0.87
Set 3	2.5	81% *Glucose*				
		19% *Acetate*	190	148	0.78	0.85
Set 4	2	49% *Glucose*				
		51% *Acetate*	950	205	0.22	0.82
Set 5	2	24% *Glucose*				
		76% *Acetate*	725	155	0.21	0.78

than that of PHB from *acetate* due to the fact that storage of glycogen, spending 0.17 ATP/C-mole is energetically more efficient than storage of PHB which spends 0.25 mole ATP/C-mole (Dircks *et al*., 2001).

Experiments on glucose/acetate mixtures. After experiments on *acetate* and *glucose*, three additional runs were conducted using *glucose/acetate* mixtures. In these runs, the reactor was seeded by biomass previously acclimatised to the mixture. The initial substrate concentration was adjusted to 150–200 mgl^{-1} COD, and the corresponding *glucose* fraction was gradually decreased from 81% to 49 and 24%. As shown in Table 4, Y_{STO} was affected by the dominant substrate fraction in the mixture, exhibiting a parallel decrease from 0.85 $gCOD(gCOD)^{-1}$ to 0.78 $gCOD(gCOD)^{-1}$. The OUR curves of two runs where *glucose* (81%) and *acetate* (76%) were adjusted as the major substrate fraction are given in Figure 5. It is interesting to note that the OUR profiles reveal two distinctly different rates, a faster utilisation rate for *glucose* dominating the initial phase of the OUR curve and partially eclipsing a continuing slower process associated with the storage of *acetate*. This confirms similar findings reported by Carta *et al*. (2001).

Experiments on domestic sewage

Four tests were conducted on *domestic sewage* at different F/M ratios varied in the range of 0.09–0.42 $gCOD(gcellCOD)^{-1}$, using the filtered portion of a daily composite sample collected from the inlet of the Ataköy plant, a small wastewater treatment facility in Istanbul treating only domestic sewage. The sample could be characterised by a total soluble COD of 100 mgl^{-1} and a soluble biodegradable fraction of 90 mgl^{-1}, based on a S_I/S_T ratio of 0.10 ascertained for the same sewage. As outlined in Table 5, a Y_{STO} of 0.96 $gCOD(gCOD)^{-1}$, a substantially higher value as compared to *acetate* and *glucose*, was

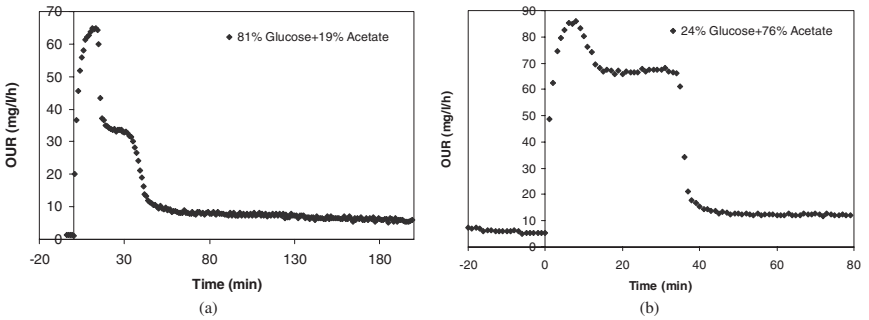

Figure 5 OUR profiles for *glucose/acetate* mixtures (a) 81% *glucose* (b) 24% *glucose*

practically applicable to all four tests. Table 5 also gives the results of three additional experiments carried out on *domestic sewage/acetate* mixtures. The results confirm the validity of Y_{STO} values individually calculated for *domestic sewage* and *acetate*, indicating a transient pattern reflecting the character of the dominant substrate fraction in the mixture.

The calculations were made with the assumption that the entire soluble biodegradable COD could be regarded as readily biodegradable substrate. This assumption, although valid for pure substrates, requires, as in this case, careful evaluation for wastewaters with more complex substrate compositions. If readily biodegradable substrate is actually less than what is indicated by the assumption introduced with ASM3, the corresponding oxygen consumption can only be interpreted with a superficially higher Y_{STO}. The proposed procedure may also be used to calculate S_{S1} for a generally adopted Y_{STO} value, as it defines a stoichiometric procedure between the storage yield and the available readily biodegradable substrate. In this context, adoption of $Y_{STO} = 0.78$ gCOD(gCOD)$^{-1}$, associated with *acetate* in this study, would yield $S_{S1} = 18$ mgl^{-1}, corresponding to a S_S/S_T ratio of 0.18. Similarly, $S_{S1} = 29$ mgl^{-1}, approximately 32% of the level that was previously assumed as readily biodegradable COD, would be obtained using the Y_{STO} value of 0.85 gCOD(gCOD)$^{-1}$ suggested in ASM3.

Conclusions

A procedure was proposed for the experimental assessment of the storage yield as defined in ASM3. The procedure makes use of the OUR curve generated by a soluble (filtered) substrate in an aerated batch reactor and enables graphical identification and calculation of the amount of oxygen consumed for storage. The biodegradable fraction of the soluble/filtered substrate must also be determined. No model simulation or evaluation is required as the suggested procedure is based on simple stoichiometry between substrate utilised and dissolved oxygen consumed. Model simulation indicated that the proposed procedure was quite accurate, involving only an error of less than 2%, aside from analytical errors

Table 5 Experimental assessment of Y_{STO} for *domestic sewage* and *domestic sewage/acetate* mixtures

Set No.	Reactor volume (l)	Composition	Biomass concentration (mg COD/l)	Substrate concentration (mg COD/l)	F/M ratio (COD/COD)	Y_{STO}
Set 1	2.5	*Domestic sewage*	580	51	0.09	0.95
Set 2	3	*Domestic sewage*	265	50	0.19	0.97
Set 3	2.5	*Domestic sewage*	145	54	0.37	0.96
Set 4	2.5	*Domestic sewage*	120	51	0.42	0.96
Set 5	3	*80% Domestic sewage+20% HAc.*	80	45	0.56	0.90
Set 6	3	*50% Domestic sewage+50% HAc.*	180	50	0.28	0.87
Set 7	3	*27% Domestic sewage+73% HAc.*	160	50	0.31	0.82

associated with standard COD measurements, for tests to be conducted with F/M ratios over 0.1 gCOD(gcellCOD)$^{-1}$.

The proposed procedure was tested on *acetate*, *glucose* and *domestic sewage*, together with mixtures of *acetate/glucose* and *acetate/domestic sewage*. Relevant experimental data, generated at different initial F/M ratios, yielded an average Y_{STO} value of 0.78 gCOD(gCOD)$^{-1}$ for *acetate*, 0.87 gCOD(gCOD)$^{-1}$ for *glucose* and 0.96 gCOD(gCOD)$^{-1}$ for *domestic sewage*. The high Y_{STO} level related to *domestic sewage*, consistently obtained for a wide range of initial F/M ratios, challenges the validity of the concept of S_S in ASM3, which is defined as the biodegradable fraction of the soluble substrate and tested in the study. The proposed procedure may also be used to calculate S_{S1} for a generally adopted Y_{STO} value, as it defines a stoichiometric procedure between the storage yield and the available readily biodegradable substrate.

The experiments conducted on substrate mixtures confirmed the validity of Y_{STO} values calculated for individual substrates, yielding a transient pattern reflecting the character of the dominant substrate fraction in the mixture. For *glucose/acetate* mixtures, they provided a clear indication of a faster storage rate for *glucose* as compared to *acetate*.

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