CALIBRATION OF ACTIVATED SLUDGE MODELS: A CRITICAL REVIEW OF EXPERIMENTAL DESIGNS

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Abstract

This review begins with an overview of literature data on methodologies that have been applied in other studies to calibrate Activated Sludge Model No. 1 (ASM1). An attempt was made to gather and summarise the information needed to achieve a successful model calibration, and based on this a general model calibration procedure is proposed. The main part of the literature review is devoted to the different methods that have been developed and applied for the characterisation of wastewater and reaction kinetics in relation to ASM1. The methodologies are critically discussed and it is attempted to illustrate the power of the different methods for characterisation, all within the frame of ASM1 calibration. Finally, it is discussed which wastewater components and parameters are most relevant to be characterised via lab-scale experiments. This discussion also includes the problem of transferability between lab-scale and full-scale observations, and potentially different model concepts. One of the most discussed experimental factors determining the experimental response is the ratio between initial substrate and biomass concentration (S(0)/X(0)). A separate section is focusing upon this factor.

1. Introduction

One of the most widespread biological wastewater treatment techniques is the activated sludge process. In this process, a bacterial biomass suspension is responsible for the removal of pollutants. Depending on the design and the specific application, an activated sludge wastewater treatment plant can achieve biological nitrogen removal and biological phosphorus removal, besides removal of organic carbon substances. The increased knowledge about the mechanisms of different biological processes taking place in an activated sludge plant was translated into dynamic models that were

developed to describe the degradation processes in the activated sludge plant. This review will focus on the Activated Sludge Model No. 1 (ASM1) (Henze *et al.*, 1987), which through the years has been the state-of-the-art model for activated sludge plants with biological nitrogen removal.

2. Description of the state-of-the-art activated sludge models

In the following the model concepts of ASM1 (Henze *et al.*, 1987) and the recent modifications leading to ASM3 (Gujer *et al.*, 1999) are described. A description of ASM2/ASM2d (Henze *et al.*, 1995, 1999) is, however, not included since phosphorus removal is not dealt with in this review.

2.1 ACTIVATED SLUDGE MODEL No.1 (ASM1)

ASM1 is presented in a matrix format in Table 1 according to Henze *et al.* (1987). Many of the basic concepts of ASM1 were adapted from the activated sludge model defined by Dold (1980). Some of the central concepts (the different model components and processes) of ASM1 are summarised below. For further details the reader is referred to the IAWQ Task group reports.

Table 1. The ASM1 process matrix (Henze et al., 1987) (cont' on next page)

	Component (i) → ↓ Process (j)	1 S_I	2 S_S	3 X_I	4 X_S	5 X_{BH}	6 X_{BA}	7 X_P	8 S_O	9 S_{NO}	10 S_{NH}
1	Aerobic growth of heterotrophic biomass		$-\frac{1}{Y_H}$			1			$-\frac{1-Y_H}{Y_H}$		$-i_{XB}$
2	Anoxic growth of heterotrophic biomass		$-\frac{1}{Y_H}$			1					$-i_{XB}$
3	Aerobic growth of autotrophic biomass						1		$-\frac{4.57-Y_A}{Y_A}$	$-\frac{1}{Y_A}$	$-i_{XB}-\frac{1}{Y_A}$
4	Decay of heterotrophic biomass				$1-f_P$	-1		f_P			
5	Decay of autotrophic biomass				$1-f_P$		-1	f_P			
6	Ammonification of soluble organic nitrogen										1
7	Hydrolysis of slowly biodegradable substrate		1		-1						
8	Hydrolysis of organic nitrogen										

2.1.1 COD components in ASM1

COD is selected as the most suitable parameter for defining the carbon substrates as it provides a link between electron equivalents in the organic substrate, the biomass and oxygen utilised. In ASM1 the COD is subdivided based on (1) solubility, (2) biodegradability (3) biodegradation rate and (4) viability (biomass):

- The total COD is divided into soluble (S) and particulate (X) components.
- The COD is further subdivided into non-biodegradable organic matter and biodegradable matter. The non-biodegradable matter is biologically inert and passes through an activated sludge system in unchanged form. The inert soluble organic matter (S_I) leaves the system at the same concentration as it enters. Inert suspended organic matter in the wastewater influent (X_I) or produced via decay (X_P) becomes enmeshed in the activated sludge and is removed from the system via the sludge wastage.
- The biodegradable matter is divided into soluble readily biodegradable (S_S) and slowly biodegradable (X_S) substrate. Already here it should be stressed that some slowly biodegradable matter may actually be soluble. The readily biodegradable substrate is assumed to consist of relatively simple molecules that may be taken in directly by heterotrophic organisms and used for growth of new biomass. On the

Table 1. The ASM1 process matrix (Henze et al., 1987) (cont' from previous page)

11 S_{ND}	12 X_{ND}	13 S_{ALK}	Process rate (ρ_j)
		$-\frac{i_{XB}}{14}$	$\mu_{max\,H} \cdot \frac{S_S}{K_S + S_S} \cdot \frac{S_O}{K_{OH} + S_O} \cdot X_{BH}$
		$\frac{1-Y_H}{14 \cdot 2.86 \cdot Y_H} - \frac{i_{XB}}{14}$	$\eta_g \cdot \mu_{max\,H} \cdot \frac{S_S}{K_S + S_S} \cdot \frac{K_{OH}}{K_{OH} + S_O} \cdot \frac{S_{NO}}{K_{NO} + S_{NO}} \cdot X_{BH}$
		$-\frac{2}{14\,Y_A} - \frac{i_{XB}}{14}$	$\mu_{max\,A} \cdot \frac{S_{NH}}{K_{NH} + S_{NH}} \cdot \frac{S_O}{K_{OA} + S_O} \cdot X_{BA}$
	$i_{XB} - f_P \cdot i_{XP}$		$b_H \cdot X_{BH}$
	$i_{XB} - f_P \cdot i_{XP}$		$b_A \cdot X_{BA}$
-1		$\frac{1}{14}$	$k_a \cdot S_{ND} \cdot X_{BH}$
			$k_h \cdot \frac{X_S / X_{BH}}{K_X + X_S / X_{BH}} \cdot \frac{S_O}{K_{OH} + S_O} + \eta_h \cdot \frac{K_{OH}}{K_{OH} + S_O} \cdot \frac{S_{NO}}{K_{NO} + S_{NO}} \cdot X_{BH}$
1	-1		$\rho_7 \cdot (X_{ND}/X_S)$

contrary, the slowly biodegradable substrate consists of relatively complex molecules that require enzymatic breakdown prior to utilisation.

- Finally, heterotrophic biomass (X_{BH}) and autotrophic biomass (X_{BA}) are generated by growth on the readily biodegradable substrate (S_S) or by growth on ammonia nitrogen (S_{NH}). The biomass is lost via the decay process where is it converted to X_P and X_S (death regeneration, see below).

Summarising, the total COD balance of ASM1 is defined by Eq. 1 and further illustrated in Fig. 1.

$$\mathrm{CODtot} = S_I + S_S + X_I + X_S + X_{BH} + X_{BA} + X_P \quad (1)$$

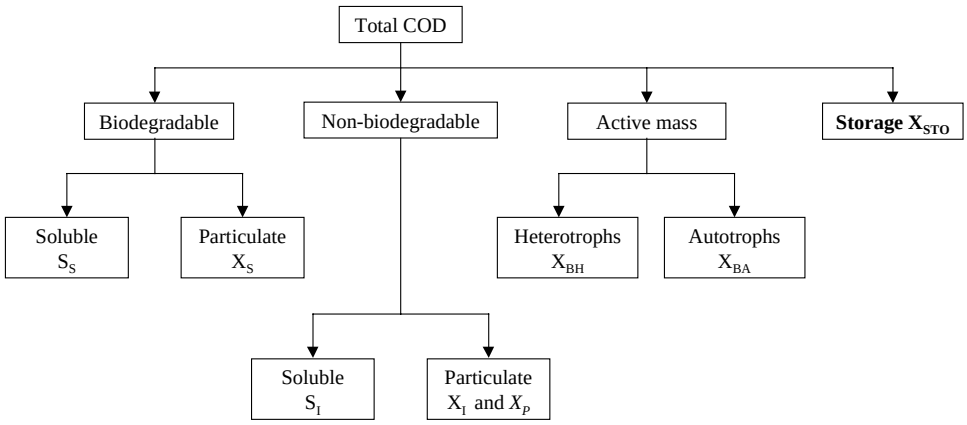

Fig 1. COD components in ASM1 and ASM3 (figure modified from Jeppsson, 1996), components specifically related to ASM3 are given in bold and the ones only related to ASM1 are given in italics

2.1.2 Nitrogen components in ASM1

Similar to the organic matter, total nitrogen can be subdivided based on (1) solubility, (2) biodegradability and (3) biodegradation rate:

- Total nitrogen can be subdivided into soluble (S) and particulate (X) components.
- The nitrogen is divided into non-biodegradable matter and biodegradable matter. The non-biodegradable particulate organic nitrogen (X_{NI}) is associated with the non-biodegradable particulate COD (X_I or X_P), whereas the soluble non-biodegradable organic nitrogen (S_{NI}) is assumed to be negligible and therefore not incorporated into the model.
- The biodegradable nitrogen is subdivided into ammonia nitrogen (S_{NH}), nitrate + nitrite nitrogen (S_{NO}), soluble organic nitrogen (S_{ND}) and particulate organic nitrogen (X_{ND}). The particulate organic nitrogen is hydrolysed to soluble organic

nitrogen in parallel with hydrolysis of the slowly biodegradable organic matter (X_S) (either present in the wastewater or produced via the decay process). The soluble organic nitrogen is converted to ammonia nitrogen via ammonification. Ammonia nitrogen serves as the nitrogen source for biomass growth (the parameter i_{XB} indicates the amount of nitrogen incorporated per COD unit). Finally, the autotrophic conversion of ammonia results in nitrate nitrogen (S_{NO}) which is considered to be a single step process in ASM1.

Summarising, the total nitrogen balance for the components in ASM1 is defined by Eq. 2 and further illustrated in Fig. 2.

$$Ntot = S_{NH} + S_{ND} + S_{NO} + X_{ND} + X_{NI} + i_{XB} \cdot (X_{BH} + X_{BA}) + i_{XP} \cdot X_P \qquad (2)$$

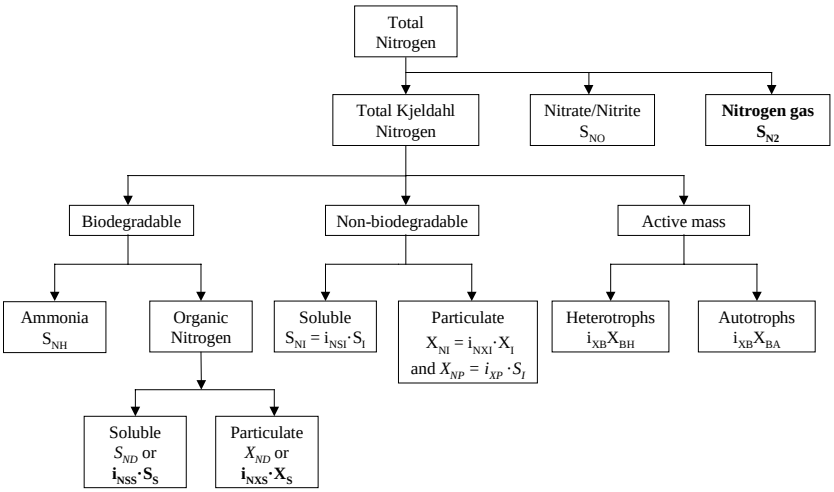

Fig 2. Nitrogen components in ASM1 (modified from Jeppsson, 1996); components specifically related to ASM3 are given in bold and the ones only related to ASM1 in italics

2.1.3 Processes in ASM1

Basically there are four different main processes defined in ASM1 (Henze *et al.*, 1987):

- Growth of biomass
- Decay of biomass
- Ammonification of organic nitrogen
- Hydrolysis of particulate organic matter

The substrate flows in ASM1 are illustrated in Fig. 3.

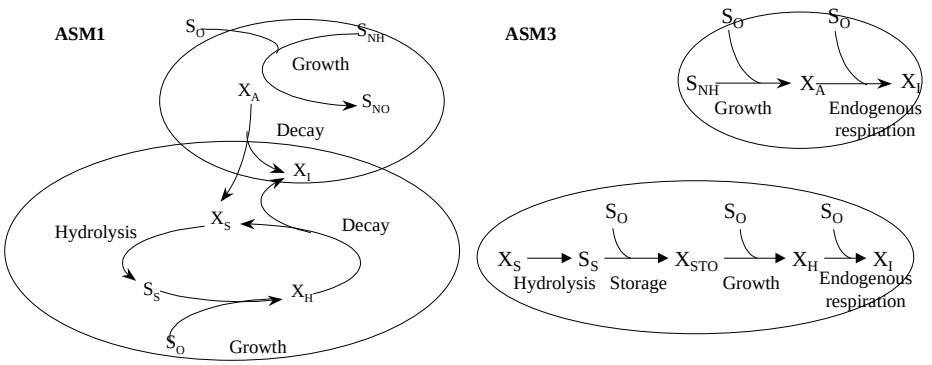

Fig 3. Substrate flows in ASM1 and ASM3 (modified from Gujer et al., 1999)

2.1.3.1. Aerobic growth of heterotrophic biomass Growth takes place by degradation of soluble readily biodegradable substrate (S_S) under the consumption of oxygen (S_O). Ammonia nitrogen (S_{NH}) is incorporated into cell mass, as described above. Both the concentrations of S_S and S_O may be rate limiting for the growth process. The Monod relationship is used to describe the growth of heterotrophic and autotrophic organisms.

2.1.3.2. Anoxic growth of heterotrophic biomass (denitrification) In the absence of oxygen the heterotrophic organisms are capable of using nitrate as the terminal electron acceptor with S_S as substrate resulting in biomass growth and nitrogen gas. The same Monod kinetics as used for aerobic growth is applied except that the kinetic rate expression is multiplied by a correction factor η_g (<1). This factor is accounting for the fact that the anoxic substrate removal rate is slower compared to aerobic conditions. This can either be caused by a lower maximum growth rate or because only a fraction of the heterotrophic biomass is able to denitrify. Furthermore, anoxic growth is inhibited when oxygen is present which is described by the switching function $K_{OH}/(K_{OH}+S_O)$. The coefficient K_{OH} has the same value as in the expression for aerobic growth. Thus, as aerobic growth declines, the capacity for anoxic growth increases.

2.1.3.3. Aerobic growth of autotrophic biomass (nitrification) Ammonia nitrogen (S_{NH}) is oxidised to nitrate resulting in production of autotrophic biomass. Furthermore, a part of the S_{NH} is also incorporated in the autotrophic cell mass. As for heterotrophic growth the concentrations of S_{NH} and S_O can be rate limiting for the process. Nitrification has a considerable effect on the alkalinity (S_{ALK}).

2.1.3.4. Decay of heterotrophic biomass The death regeneration concept of Dold (1980) is applied to describe the different reactions that take place when organisms die. The traditional endogenous respiration concept describes how a fraction of the organism mass disappears to provide energy for maintenance. However, in the death regeneration concept oxygen is not directly associated with microbial decay. Decay is assumed to result in the release of slowly biodegradable substrate that is recycled back to soluble substrate and used for more cell growth. Thus, the oxygen utilisation normally

associated directly with decay is calculated as if it occurs indirectly from growth of new biomass on released substrate. A parallel conversion of organic nitrogen to ammonia nitrogen occurs. It should be noted that the magnitude of the decay coefficient used in this approach is different from that of the endogenous respiration. In endogenous respiration the loss of one unit of biomass COD leads to the utilisation of one unit of oxygen minus the COD of the inert particulate products that are formed. However, in the death regeneration model the loss of one biomass COD unit results in the ultimate formation of one unit of COD due to the formed readily biodegradable substrate minus the formed inert particulate products. When the readily biodegradable COD is used for cell synthesis, only a fraction of a unit of oxygen (determined by the yield) will be required because of the energy incorporated into the cell mass. That cell mass undergoes in turn decay etc. before the unit of oxygen is finally removed.

Summarising, to give the same amount of oxygen utilisation per time due to the decay process, the decay rate coefficient must be larger for the death regeneration concept than if a more traditional endogenous decay process was adopted. This has the effect that the cell mass turnover rate increases, resulting in a higher microbial growth rate in the death regeneration model.

2.1.3.5. Decay of autotrophic biomass The decay of autotrophs is described similar to the heterotrophic decay process.

2.1.3.6. Ammonification of soluble organic nitrogen (S_{ND}) Biodegradable soluble organic nitrogen (S_{ND}) is converted to ammonia nitrogen (S_{NH}) in a first order process. Hydrogen ions consumed in this conversion process result in an alkalinity change.

2.1.3.7. Hydrolysis Slowly biodegradable substrate (X_S) enmeshed in the sludge is broken down producing readily biodegradable substrate (S_S). The degradation of slowly biodegradable matter has appeared rather important to realistic modelling of activated sludge systems because it is primarily responsible for realistic electron acceptor profiles (Dold, 1980). This process is modelled on the basis of surface reaction kinetics and occurs only under aerobic and anoxic conditions. The hydrolysis rate is reduced under anoxic conditions in the same way as anoxic growth, by applying a correction factor η_h (<1). The rate is also first order with respect to the heterotrophic biomass concentration present but saturates, as the amount of entrapped substrate becomes large in proportion to the biomass.

2.1.4 Restrictions of ASM1

A number of restrictions concerning ASM1 are summarised below (Henze *et al.*, 1987):

- The system must operate at constant temperature.
- The pH is constant and near neutrality. It is known that the pH has an influence on many of the parameters, however only limited knowledge is available to be able to express these possible influences. Consequently, a constant pH has been assumed. The inclusion of alkalinity in the model, however, does allow for detection of pH problems.

- No considerations have been given to changes in the nature of the organic matter within any given wastewater fractions (e.g. the readily biodegradable substrate). Therefore, the parameters in the rate expressions have been assumed to have constant values. This means that only concentration changes of the wastewater components can be handled whereas changes in the wastewater character can not.
- The effects of nutrient limitations (e.g. N and P) on the cell growth have not been considered. It is, however, easy to add limitation terms in the model if needed.
- The correction factors for denitrification (η_g and η_h) are fixed and constant for a given wastewater, even though it is possible that their values are depending on the system configuration.
- The parameters for nitrification are assumed to be constant and to incorporate any inhibitory effects that wastewater constituents may have on them.
- The heterotrophic biomass is homogeneous and does not undergo changes in species diversity with time. This assumption is inherent to the assumption of constant kinetic parameters. This means that any changes in substrate concentration gradients, reactor configuration, etc. on sludge settleability are not considered.
- The entrapment of particulate organic matter in the biomass is assumed to be instantaneous.
- The hydrolysis of organic matter and organic nitrogen are coupled and occur simultaneously with equal rates.
- The type of electron acceptor present does not affect the loss of biomass by decay.
- The type of electron acceptor does not affect the heterotrophic yield coefficient.
- ASM1 is developed for simulation of treatment of municipal wastewater, and it is therefore not advised to apply the model to systems where industrial contributions dominate the characteristics of the wastewater.
- ASM1 does not include processes that describe behaviours under anaerobic conditions. Simulations of systems with large fractions of anaerobic reactor volume may therefore lead to errors.
- ASM1 can not deal with elevated nitrite concentrations.
- ASM1 is not designed to deal with activated sludge systems with very high load or small sludge retention time (SRT) (<1 day).

2.2 ACTIVATED SLUDGE MODEL NO. 3 (ASM3)

ASM3 is presented in matrix form in Table 2. In the development of ASM3 some limitations of ASM1 were evaluated, and combined with the experiences gained with the application of ASM1 the following list of “defects” of ASM1 was defined (Gujer *et al.*, 1999):

- ASM1 does not include expressions to deal with nitrogen and alkalinity limitations.
- ASM1 considers biodegradable soluble and particulate organic nitrogen as model components. These can, however, not easily be measured and may in most cases unnecessarily complicate the use of ASM1.
- The ammonification kinetics can not be easily quantified, and moreover this process is typically rather fast and does therefore not affect model predictions significantly.

Table 2. Rate expressions and stoichiometry of ASM3 (Gujer et al., 1999)

j	Process	Process rate equation ρ_j, all $\rho_j \geq 0$
1	Hydrolysis	$k_H \cdot \frac{X_S / X_H}{K_X + X_S / X_H} \cdot X_H$
Heterotrophic organisms, aerobic and denitrifying activity		
2	Aerobic storage of S_S	$k_{STO} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot \frac{S_S}{K_S + S_S} \cdot X_H$
3	Anoxic storage of S_S	$k_{STO} \cdot \eta_{NOX} \cdot \frac{K_{O2}}{K_{O2} + S_{O2}} \cdot \frac{S_{NOX}}{K_{NOX} + S_{NOX}} \cdot \frac{S_S}{K_S + S_S} \cdot X_H$
4	Aerobic growth	$\mu_H \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot \frac{S_{NH4}}{K_{NH4} + S_{NH4}} \cdot \frac{S_{ALK}}{K_{ALK} + S_{ALK}} \cdot \frac{X_{STO} / X_H}{K_{STO} + X_{STO} / X_H} \cdot X_H$
5	Anoxic growth (denitrification)	$\mu_H \cdot \eta_{NOX} \cdot \frac{K_{O2}}{K_{O2} + S_{O2}} \cdot \frac{S_{NOX}}{K_{NOX} + S_{NOX}} \cdot \frac{S_{NH4}}{K_{NH4} + S_{NH4}} \cdot \frac{S_{ALK}}{K_{ALK} + S_{ALK}} \cdot \frac{X_{STO} / X_H}{K_{STO} + X_{STO} / X_H} \cdot X_H$
6	Aerobic endogenous respiration	$b_{H,O2} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot X_H$
7	Anoxic endogenous respiration	$b_{H,NOX} \cdot \frac{K_{O2}}{K_{O2} + S_{O2}} \cdot \frac{S_{NOX}}{K_{NOX} + S_{NOX}} \cdot X_H$
8	Aerobic respiration of X_{STO}	$b_{STO,O2} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot X_{STO}$
9	Anoxic respiration of X_{STO}	$b_{STO,NOX} \cdot \frac{K_{O2}}{K_{O2} + S_{O2}} \cdot \frac{S_{NOX}}{K_{NOX} + S_{NOX}} \cdot X_{STO}$
Autotrophic organisms, nitrifying activity		
10	Aerobic growth of X_A, Nitrification	$\mu_A \cdot \frac{S_{O2}}{K_{A,O2} + S_{O2}} \cdot \frac{S_{NH4}}{K_{A,NH4} + S_{NH4}} \cdot \frac{S_{ALK}}{K_{A,ALK} + S_{ALK}} \cdot X_A$
11	Aerobic endogenous respiration	$b_{A,O2} \cdot \frac{S_{O2}}{K_{A,O2} + S_{O2}} \cdot X_A$
12	Anoxic endogenous respiration	$b_{A,NOX} \cdot \frac{K_{A,O2}}{K_{A,O2} + S_{O2}} \cdot \frac{S_{NOX}}{K_{A,NOX} + S_{NOX}} \cdot X_A$

j ∨	Process	compound i > 1 S_{O2}	2 S_I	3 S_S	4 S_{NH4}	5 S_{N2}	6 S_{NOX}	7 S_{ALK}	8 X_I	9 X_S	10 X_H	11 X_{STO}	12 X_A	13 X_{SS}
	expressed as >	O_2	COD	COD	N	N	N	Mole	COD	COD	COD	COD	COD	SS
1	Hydrolysis		f_{SI}	1	i			0.001		-1				-0.75
Heterotrophic organisms, aerobic and denitrifying activity														
2	Aerobic storage of S_S	1-$Y_{STO,2}$		-1	0.03			0.002				0.85		0.51
3	Anoxic storage of S_S			-1	0.03	0.07	-0.07	0.007				0.80		0.48
4	Aerobic growth	-0.60			-0.07			-0.005			1	-1.60		-0.06
5	Anoxic growth (denitrific.)				-0.07	0.30	-0.30	0.016			1	-1.85		-0.21
6	Aerobic endog. respiration	-0.80			0.066			0.005	0.20		-1			-0.75
7	Anoxic endog. respiration				0.066	0.28	-0.28	0.025	0.20		-1			-0.75
8	Aerobic respiration of X_{STO}	-1										-1		-0.60
9	Anoxic respiration of X_{STO}					0.35	-0.35	0.025				-1		-0.60
Autotrophic organisms, nitrifying activity														
10	Aerobic growth of X_A	-18.04			-4.24		4.17	-0.600					1	0.90
11	Aerobic endog. respiration	-0.80			0.066			0.005	0.20				-1	-0.75
12	Anoxic endog. respiration				0.066	0.28	-0.28	0.025	0.20				-1	-0.75

- ASM1 differentiates between inert suspended organic matter present in the influent wastewater and produced within the activated sludge process. In reality, however, it is impossible to distinguish between these two components.
- Hydrolysis has a rather dominating effect upon the predictions of the oxygen consumption and denitrification by heterotrophic organisms. In reality this process includes different coupled processes such as hydrolysis, lysis and storage of substrates. Therefore, the identification of the kinetic parameters of this combined process is difficult.
- The death regeneration concept is covering lysis combined with hydrolysis of released substrate and subsequently growth on this substrate. In reality it is difficult to determine the decay coefficient related to the death regeneration concept.
- Elevated concentrations of readily biodegradable organic substrates can lead to storage of poly-hydroxy-alkanoates, lipids or glycogen. This process is not included in ASM1.
- ASM1 does not include the possibility to differentiate between decay rates of nitrifiers under aerobic and anoxic conditions. This may lead to problems with the predictions of the maximum nitrification rates in cases of high SRT and high fractions of anoxic reactor volumes.

The main difference between ASM1 and ASM3 is the recognition of the importance of storage polymers in the heterotrophic conversions in the activated sludge processes in ASM3. The aerobic storage process in ASM3 describes the storage of the readily biodegradable substrate (S_S) into a cell internal component (X_{STO}). This approach requires that the biomass is modelled with cell internal structure similar to ASM2. The energy required for this process is obtained via aerobic respiration. This internal component is then subsequently used for growth. In ASM3 it is assumed that all S_S is first taken up and stored prior to growth. Thus, a division of the storage and growth process, allowing growth to take place on external substrate directly, is not considered.

Furthermore, the death regeneration concept is replaced by endogenous respiration, which is closer to the phenomena observed in reality. Endogenous respiration can readily be obtained from a simple batch test (see below, section 4.1.3.1). Also, ASM3 allows a differentiation between aerobic and anoxic decay.

Fig. 3 illustrates the difference in COD flows between ASM1 and ASM3. The first thing to notice is that the conversion processes of both groups of organisms (autotrophs and heterotrophs) are clearly separated in ASM3, whereas the decay regeneration cycles of the autotrophs and heterotrophs are strongly interrelated in ASM1. This change of decay concept (and introduction of the storage step) means that there exist more “entry” points for oxygen utilisation resulting in, at some points, easier separation and characterisation of the processes. Second, there is a shift of emphasis from hydrolysis to storage of organic matters. This gives a change in how wastewater characterisation should be defined since the separation between S_S and X_S now should be based on the storage process rather than on the growth process. Still, the separation remains somewhat based on biodegradation rates. In ASM3 hydrolysis is obviously of a less dominating importance for the rates of oxygen consumption since only hydrolysis of X_S in the influent is considered.

Below the components and processes of AMS3 are summarised focusing on the differences between ASM1 and ASM3.

2.2.1 COD components in ASM3

The COD components in ASM3 are basically defined in the same way as in ASM1. Only the separation between inert suspended organic matter in the wastewater influent (X_I) and produced via the decay process (X_P) is no longer maintained, and, second, the component X_{STO} is introduced, as described above. The substrate S_S goes through the storage process but is basically still biodegradable. Thus, the total COD balance is defined by Eq. 3 and further illustrated in Fig. 1, where the components specifically related to ASM3 are given in bold and the ones only related to ASM1 are given in italics.

$$\mathrm{CODtot} = S_I + S_S + X_I + X_S + X_H + X_A + X_{STO} \quad (3)$$

2.2.2 Nitrogen components in ASM3

The nitrogen balance in ASM3 is simplified compared to ASM1, since the soluble and particulate organic nitrogen components are no longer considered. Furthermore, a nitrogen gas component (S_{N2}) is included allowing for a closed nitrogen mass balance. The nitrogen incorporated in S_I, S_S, X_I, X_S, and the biomass is defined in ASM3 as a fraction of these components. This fraction is consumed or produced when the corresponding COD fraction is formed or degraded respectively. Summarising, the total nitrogen balance for the components in ASM3 is defined by Eq. 4, and further illustrated in Fig. 2. Again, the components specifically related to ASM3 are shown in bold and the ones related to ASM1 in italics.

$$\mathrm{Ntot} = S_{NH} + S_{NO} + S_{N2} + i_{NSI} \cdot S_I + i_{NSS} \cdot S_S + i_{NXS} \cdot X_S + i_{NBM} \cdot (X_H + X_A) + i_{NXI} \cdot X_I \quad (4)$$

2.2.3 Processes in ASM3

In ASM3 there are also four basic processes, however, slightly different from ASM1 (Gujer *et al.*, 1999):

- Storage of readily biodegradable substrate
- Growth of biomass
- Decay of biomass
- Hydrolysis of particulate organic matter

2.2.3.1. Aerobic storage of readily biodegradable substrate This process describes the storage of readily biodegradable substrate (S_S) in the form of X_{STO} with the consumption of oxygen. As stated above, it is assumed that all S_S first becomes stored material before use for cell growth. It is realised that this is not in accordance with reality. However, no model is currently available to predict the separation of S_S into

direct growth and storage. Gujer *et al.* (1999) therefore suggested to apply a low storage yield (Y_{STO}) and a higher growth yield (Y_H) to approximate direct growth.

2.2.3.2. Anoxic storage of readily biodegradable substrate This process is identical to the aerobic storage, only is nitrate used as terminal electron acceptor instead of oxygen. Furthermore, a correction factor (η_{NO}) is applied to indicate that only a fraction of the heterotrophic biomass may be capable of denitrifying.

2.2.3.3. Aerobic growth of heterotrophs Aerobic heterotrophic growth takes place by degradation of X_{STO} with the consumption of oxygen (S_O). Ammonia nitrogen (S_{NH}) is incorporated into cell mass, as described above for ASM1.

2.2.3.4. Anoxic growth of heterotrophs (denitrification) Anoxic growth is similar to aerobic growth but respiration is based on denitrification. Again, a correction factor (η_{NO}) is applied to account for the observation of reduced anoxic respiration rates compared to aerobic respiration.

2.2.3.5. Aerobic growth of autotrophs (nitrification) This process is described similar to ASM1.

2.2.3.6. Aerobic decay of heterotrophs The energy requirements not associated with growth but including maintenance, lysis, etc. are described by endogenous respiration in ASM3 according to a simple first order reaction kinetics.

2.2.3.7. Anoxic decay of heterotrophs ASM3 allows for a description of anoxic decay in a similar way as the aerobic decay process.

2.2.3.8. Aerobic and anoxic decay of autotrophs The decay of autotrophs is described in the same way as the heterotrophic decay process.

2.2.3.9. Aerobic and anoxic respiration of storage products These processes are analogous to endogenous respiration and ensure that the storage product X_{STO} decays together with the biomass.

2.2.3.10. Hydrolysis Just as in ASM1 hydrolysis is responsible for the breakdown of slowly biodegradable substrate (X_S) to readily biodegradable substrate (S_S). However, in ASM3 hydrolysis is assumed to be electron donor independent, and as stressed above the hydrolysis does not play the same dominating role as in ASM1.

2.2.4 Restrictions of ASM3

The number of restrictions listed for ASM1 above (see 2.1.4) basically still holds for ASM3, except for the restriction stating that the type of electron acceptor does not affect the biomass decay.

3. Model Calibration

In this review model calibration is understood as the adaptation of a model to fit a certain set of information obtained from the full-scale WWTP under study. This task is often rather time-consuming, and typically the time needed for a model calibration is underestimated. Even though more than a decade has passed since the publication of ASM1, a fully developed model calibration procedure has not been defined yet. We have not been able to find a complete model calibration report in literature. There may be many reasons for this. Important to realise is that the purpose of a model being built is very much determining on how to approach the calibration, making it difficult to generalise (Henze *et al.*, 1995). Still, considering the wide application of the activated sludge models there are surprisingly few references that contain details on the applied model calibration procedure. Most often it is not specified in detail how the model was calibrated but the focus is more on the applications, e.g. for process scenarios and optimisations etc. Thus, to obtain information on model calibration procedures one often has to collect bits and pieces from various sources to obtain an overview.

Before going on with a discussion on how to approach a model calibration of ASM1, it is relevant to define how parameter estimation is understood in this review and what the difference is between parameter estimation and model calibration. Furthermore, the term identifiability will be defined and the problem of identifiability with respect to ASM in general will be addressed.

Parameter estimation consists of determining the "optimal" values of the parameters of a given model with the aid of measured data. Here, the numerical techniques for estimation will not be discussed, but reference is made to the literature (Robinson, 1985; Vanrolleghem and Dochain, 1998). Only the basic idea behind parameter estimation is schematised in Fig. 4. Initially, the model structures, of which selected parameters need to be estimated, and the experimental data need to be defined. Moreover, first guesses of the initial conditions, i.e. concentrations, and parameters, have to be given. The parameter estimation routine then basically consists of minimising an objective function, which for example can be defined as the weighted sum of squared errors between the model output and the data. When the objective function reaches a minimum with a certain given accuracy the optimal parameter values are obtained.

Thus, parameter estimation is carried out via specific mathematical search algorithms. However, due to the high complexity caused by the numerous parameters and the unidentifiable nature of the ASM models, it will be rather cumbersome to apply mathematical calibration techniques.

Indeed, a major problem encountered in calibration of ASM is the (lack of) identifiability of the model parameters. Identifiability is the ability to obtain a unique combination of parameters describing a system behaviour. A distinction should be made between theoretical and practical identifiability. Theoretical identifiability is a property of the model structure, and relates to the question whether it is at all possible to obtain unique parameter values for a given model structure considering certain selected outputs, and assuming ideal measurements. Practical identifiability, on the other hand, includes the quality of the data. Thus, theoretically identifiable parameters may be practically unidentifiable if the data are too noise corrupted (Holmberg, 1982; Jeppsson, 1996). This subject is dealt with in great detail in Petersen (2000).

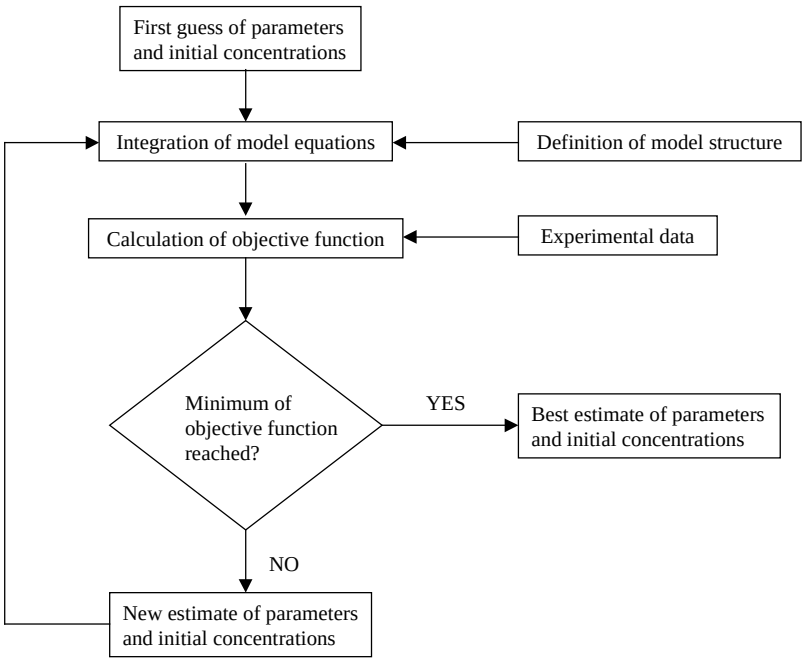

Fig 4. Illustration of parameter estimation routine (modified from Wanner et al., 1992)

Here, it should only be stressed that a typical problem related to the model calibration of ASM is that more than one combination of influent characteristics and model parameters can give the same good description of the collected data (Dupont and Sinkjær, 1994, Kristensen *et al.*, 1998). Indeed, this indicates identifiability problems of either theoretical or practical origin.

The model calibration of ASM is typically based on a step-wise procedure, and by changing just a few of the many parameters instead of applying an automatic mathematical optimisation routine. Based on the above statements concerning identifiability problems it is, however, obvious that a calibration procedure where the model parameters are changed by trial and error until a good description of the measured data is reached is not advisable (Dupont and Sinkjær, 1994, Kristensen *et al.*, 1998). Thus, it becomes important to gather as much information as possible that can help the framing of realistic parameter combinations. In this review it was attempted to gather and summarise the type of information needed for successful model calibration.

3.1 INFORMATION SET FOR MODEL CALIBRATION

The set of information that should be collected for successful model calibration was extracted and combined from different sources (Henze *et al.*, 1987; Henze, 1992; Lesouef *et al.*, 1992; Pedersen and Sinkjær, 1992; Siegrist and Tschui, 1992; Stokes *et al.*, 1993; de la Sota *et al.*, 1994; Dupont and Sinkjær, 1994; Funamizu and Takakuwa,

1994; Weijers *et al.*, 1996; Xu and Hultman, 1996; Coen *et al.*, 1997; Mino *et al.*, 1997; Kristensen *et al.*, 1998), and is summarised below:

- Design data: reactor volumes, pump flows and aeration capacities.
- Operational data:
  - Flow rates, as averages or dynamic trajectories, of influent, effluent, recycle and waste flows.
  - pH, aeration and temperatures.
- Characterisation for the hydraulic model, e.g. the results of tracer tests.
- Characterisation for the settler model: e.g. zone settling velocities at different mixed liquor suspended solids concentrations.
- Characterisation for the biological model, ASM, of:
  - Wastewater concentrations of full-scale WWTP influent and effluent (as well as some intermediate streams between the WWTP's unit processes), as averages or as dynamic trajectories: e.g. SS, COD, TKN, NH_4-N, NO_3-N, PO_4-P etc.
  - Sludge composition: e.g. SS, VSS, COD, N and/or P content.
  - Reaction kinetics: e.g. growth and decay rates.
  - Reaction stoichiometry: e.g. biomass yields

The list does not discuss on how the particular information can be collected in practice, since this will be discussed more in detail in the sections below.

As mentioned above, the required quality and quantity of information will depend very much on the purpose of the modelling exercise. In case the model is to be used for educational purposes (e.g. to increase basic understanding of the processes), for comparison of design alternatives for non-existing plants or in other situations where qualitative comparisons are sufficient, the default parameter values defined by Henze *et al.* (1987) can be applied. A reasonably good description can most often be obtained with this default parameter set for typical municipal cases without significant industrial influences (Henze *et al.*, 1997). However, if the calibrated model is going to be used for process performance evaluation and optimisation, it may be necessary to have a more accurate description of the actual processes under study. Some processes may need a more adequate description than others depending on the purpose of the model calibration. This may especially apply for models that are supposed to describe the processes in an industrial or combined municipal and industrial treatment plant (Coen *et al.*, 1997, 1998). In such cases the wastewater characterisation, and thereby the activated sludge, may differ significantly from standard municipal wastewater. In addition, special attention often has to be paid to the characterisation of nitrification kinetics (e.g. Dupont and Sinkjær, 1994), since nitrification typically is the determining process for the process designs. Also, the availability of readily biodegradable carbon substances is important for the successful achievement of both denitrification and biological P removal, and may need to be characterised in more detail (Coen *et al.*, 1997).

In this review the focus will mainly be on the information needed for the biological model. Although not considered in detail, it should be stressed that the information listed in the first 4 points is also very essential, and should not be neglected for a

successful model calibration. Major calibration problems can, for example, be related to rather simple errors in the recording of operational data, e.g. erroneous data of the waste sludge measurements might result in an incorrect sludge balance (Melcer, 1999). Moreover good characterisation of hydraulics and settling can be of great importance since e.g. poor or erroneous hydraulic modelling may result in hydraulic effects being lumped into the biological parameters of ASM1.

The information needed for the characterisation of the biological model can basically be gathered from three sources:

- Default values from literature (e.g. Henze *et al.*, 1987).
- Full-scale plant data
  - Average or dynamic data from grab or time/flow proportional samples.
  - Conventional mass balances of the full-scale data.
  - On-line data.
  - Measurements in reactors to characterise process dynamics (mainly relevant for SBR's and alternating systems).
- Information obtained from different kinds of lab-scale experiments with wastewater and activated sludge from the full-scale plant under study.

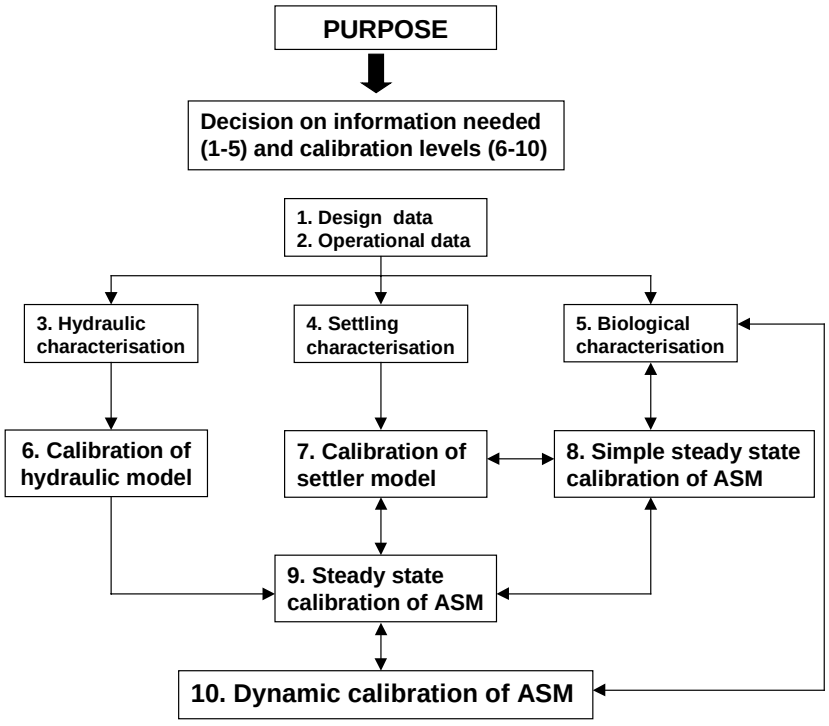

Fig 5. Schematic overview of the different general steps in an activated sludge model calibration procedure

Again, the intended use of the model will determine which information source to choose for the characterisation of the different biological processes in the model. In addition, the purpose will decide to which level the model has to be calibrated, since the quality of the desired model predictions will depend strongly on the quality of the model calibration. Fig. 5 illustrates the different general steps in a model calibration exercise. It should be stressed that not all steps may have to be taken, depending on the purpose. This will be discussed further with examples below, and the procedure has been concretised for a municipal-industrial case study in Petersen (2000).

3.2 MODEL CALIBRATION LEVELS

Steps 1-5 in Fig. 5 indicate the collection of information. Design (1) and operational (2) data are in general always needed for a model calibration. E.g. the flow and load variations are important in the design of measuring campaigns for hydraulic, sludge settling and biological characterisation of the full-scale WWTP. The hydraulics (3) are typically characterised via tracer tests at the full-scale installation (De Clercq *et al.*, 1999). The settling properties (4) can be characterised via on-line or lab-scale settling tests (Vanderhasselt *et al.*, 1999). Finally, the biology can be characterised via different information sources (see below).

In Fig. 5 steps 6-10 illustrate different calibration levels. The calibration of the hydraulic model via tracer test results, and the settler model calibration via results from sludge settling tests are indicated in steps 6 and 7 respectively. A first ASM calibration level is typically a simple steady state model calibration (8).

3.2.1 Steady state model calibration

In this step data obtained from the full-scale WWTP are averaged, thereby assuming that this average represents a steady state, and a simple model not including hydraulic detail is calibrated to average effluent and sludge waste data. Typically, the calibrations of the ASM and the settler are linked together, since the aim is most often to describe the final effluent quality. Moreover, the recycle from the settler has an influence on the activated sludge system. Thus, at this stage, there may be an interaction between the steady state calibration and the settler model calibration, indicated in Fig. 5 with the double arrow. Finally, the characterisation of wastewater components may be adjusted according to the calibration of the full-scale model, indicated with the double arrow between (8) and (5) in Fig. 5.

The next step in the calibration procedure is a steady state model calibration that includes the hydraulic model (9). In general, with a steady state model calibration, only parameters responsible for long-term behaviour of the WWTP can be determined, i.e. Y_H, f_p, b_H and X_I in the influent (Henze *et al.*, 1999; Nowak *et al.*, 1999). These parameters are correlated to a certain degree, meaning that a modification of one parameter value can be compensated by a modification of another parameter value. In the study of Nowak *et al.* (1999) on mass balances of full-scale data, it was therefore chosen to fix Y_H and f_p, leaving X_I in the influent and b_H to be determined from the steady state data. In the study of Lesouef *et al.* (1992), two WWTP models were calibrated via steady state calibration only, and this calibrated model was applied to simulate dynamic process scenarios. However, if one relies entirely on a steady state

calibration some problems may be encountered since the real input variations are usually faster than the slow process dynamics that were focused upon during the steady state calibration. In other words, the process does not operate in steady state but one still attempts to fit a steady state simplification of the model to an unsteady situation. A steady state calibration is, however, very useful for the determination of initial conditions prior to a dynamic model calibration and for the initiation of first parameter iteration (e.g. Pedersen and Sinkjær, 1992; Stokes *et al.*, 1993; Dupont and Sinkjær, 1994; Xu and Hultman, 1996; Kristensen *et al.*, 1998).

3.2.2 Dynamic model calibration

If it is the aim to describe and predict more short-term and dynamic situations, a model calibration to dynamic data will be needed since such data contain more information

Table 3. Information sources for model calibration of ASM1 (cont' on next page)

Reference	Purpose	Calibration strategy	Characterisation			
			Wastewater			
			Full-scale data	Model components		
				Mass balances	Lab-scale	Model calibration
ST92	Description: Nitrification, COD removal	Steady state n.i. Dynamic	3,7,8,9	S_I	X_I	
L92	Optimisation: N-removal	Steady state	3,4,8,9	S_S, X_S, X_{BH}	S_I, X_I	
PS92	Description: N-removal	Steady state Dynamic	3,4,5,7,8, 9	S_S, S_I, X_I		
DS94	Optimisation: N-removal	Steady state Dynamic	3,4,5,7,8, 9	n.i.		
S93	Description: Nitrification, COD removal	Steady state Dynamic	1,3,5,6,8	n.i.		
dS94	Optimisation: All processes	Steady state Dynamic	3,5,7,8,9, 10			all
XH96	Description: COD removal, N removal	Steady state Dynamic	3,4,6,8,9	S_I, S_S	S_S, X_{BH}, X_S	X_I
K98	Description: COD removal, N removal	Steady state Dynamic	1,2,3,4,7, 8,9		S_S	

n.i.: procedure not described in detail, but probably carried out.

1. SS : Suspended Solids
2. VSS : Volatile Suspended Solids
3. CODtot : total COD
4. CODsol : soluble COD
5. BOD_5 : Biological Oxygen Demand (5days)
6. TN : Total Nitrogen
7. TKN : Kjeldahl Nitrogen
8. NH_4-N : Ammonium Nitrogen
9. NO_x-N : Nitrate + Nitrite Nitrogen
10. PO_4-P : Ortho-phosphate

than steady state data, especially on fast dynamic behaviour. The important point in model calibration based on dynamic data is to obtain a more reliable estimation of the maximum specific growth rates μ_{maxH} and μ_{maxA} (Henze *et al.*, 1999), which are the most important parameters in predicting dynamic situations.

At WWTP's data are most often collected routinely with a daily or weekly sampling frequency. This sampling frequency may, however, not be high enough, and for more accurate modelling it may therefore be required to run special measuring campaigns (e.g. Pedersen and Sinkjær 1992; Dupont and Sinkjær, 1994; de la Sota *et al.*, 1994; Xu and Hultman, 1996). The sampling frequencies should be chosen in relation to the time constants of the process and influent variations. One of the important time constants of the process is the hydraulic retention time (HRT). Ideally, one should choose to sample about five times faster than the hydraulic retention time and have a test duration of 3-4 times this key time constant (Ljung, 1987). However, since measurements on full-scale

Table 3. Information sources for model calibration of ASM1 (cont' from previous page)

Characterisation		
Sludge	Kinetic and stoichiometric	
Lab-scale analyses	Model calibration	Lab-scale experiments
	Y_H, μ_H, K_S, k_h, K_X, b_H, μ_A, K_{NH}	
1,2,3,7	K_X, k_h	μ_A, b_A
	μ_A, K_S, η_g	
n.i.	K_S, η_g	μ_A, b_A, K_{NH}, K_{OA}
1,2,3	μ_H, K_S, μ_A	
1,2,3,7	μ_A, b_A, μ_H, K_S, k_h, K_X, K_{NO}, η_g, η_h,	
1	μ_A, K_{OH}, K_{NH}, η_g	
1,2	k_h, K_X, b_H, η_g, K_{OH}	b_H, μ_H, μ_A, K_{OA}

ST92	Siegrist and Tshui (1992)	dS94	de la Sota *et al.* (1994)
L92	Lesouef *et al.* (1992)	S93	Stokes *et al.* (1993)
K98	Kristensen *et al.* (1998)	DS94	Dupont and Sinkjær (1994)
XH96	Xu and Hultman (1996)	PS92	Pedersen and Sinkjær (1992)

WWTP's are relatively expensive these recommendations may not always be completely fulfilled.

Furthermore, data from the full-scale installation alone may be insufficient for a dynamic model calibration since the reaction kinetics can not be readily obtained from such data, except for specific designs like SBR's and alternating systems (Vanrolleghem and Coen, 1995). For a dynamic model calibration on a full-scale WWTP the modeller is therefore typically aiming at combining more information rich results derived from lab-scale experiments (carried out with sludge and wastewater from the full-scale installation) with data obtained from measuring campaigns on the WWTP under study (Dupont and Sinkjær, 1994; Xu and Hultman, 1996; Kristensen *et al.*, 1998).

In Table 3 an attempt is made to gather and summarise the available literature examples on model calibrations where detailed information is given on the model calibration procedures. The table should not be regarded as a complete list of possibilities but can serve as a starting point. The purpose of the different model calibrations is given together with the applied calibration strategy. Furthermore, the information sources for the characterisation of (1) wastewater, (2) sludge, (3) kinetics and (4) stoichiometry, are listed. Table 3 does not indicate the kind of experiments that may have been carried out to gather the information, since this will be discussed in one of the next sections of this review. The model parameters that are not mentioned in Table 3 have either been taken from literature or their origin may not have been clearly indicated in the references. Considering wastewater characterisation it is not always specified how the wastewater information was converted into the wastewater components according to ASM1. In these cases only the type of measurement (e.g. COD, TKN etc.) is listed in the Table.

Based on Table 3, it is obvious that the choice of information needed for the model calibration is governed by the purpose. E.g. in the studies of Pedersen and Sinkjær (1992) and Dupont and Sinkjær (1994) the emphasis was to have a description of the nitrification and denitrification, and the model calibrations therefore focused on adjustment of the parameters related to these processes. In contrast, other studies aimed at a description of both COD and N removal, and as a result more parameters had to be considered for adjustment in the model calibration (Siegrist and Tschui, 1992; de la Sota *et al.*, 1994; Xu and Hultman, 1996; Kristensen *et al.*, 1998).

The wastewater characterisation has both been carried out via full-scale data combined with mass balances and via lab-scale experiments, e.g. for the inert components S_I and X_I (Lesouef *et al.*, 1992) and the S_S component (Xu and Hultman, 1996; Kristensen *et al.*, 1998). In one study all wastewater components were determined via calibration on the full-scale data (de la Sota *et al.*, 1994). The determination of the stoichiometric and kinetic parameters is often carried out via calibration of the model to the full-scale data only. However, some studies have also included the effort of characterising some parameters in lab-scale experiments, e.g. for the determination of the specific growth rate of the autotrophic biomass (e.g. Lesouef *et al.*, 1992; Dupont and Sinkjær, 1994) or to collect further information on the half-saturation coefficients (Kristensen *et al.*, 1998).

In addition, Table 3 indicates that if the purpose of the model calibration was more than "just" a description of the processes, more emphasis was put on the characterisation of the relevant parameters via lab-scale experiments. For example in the

study of Dupont and Sinkjær (1994) the aim was to apply the model for optimisation of nitrogen removal.

Finally, Table 4 aims at summarising the most relevant parameters to adjust in the steady state and dynamic model calibration. The parameters related to the hydrolysis process are not included in Table 4. This was done on purpose since it was not clear from the literature whether the parameters of this process are most influential to short- or long-term treatment plant behaviour.

Table 4. Most relevant parameters in steady state and dynamic model calibration.

	Steady state calibration	Dynamic calibration
Predictions	Long-term	Short-term
Main relevant parameters	Y_H, f_P, b_H, $X_{I,influent}$	μ_{maxH}, μ_{maxA}, η_g, η_h, K_S, K_{NH}, K_{OH}, K_{OA}

4. Characterisation of wastewater and sludge kinetics

Different methods may be proposed to structure the wealth of methods that have been developed and applied for the characterisation of wastewater and reaction kinetics in relation to ASM1. At this point it is assumed that the reader is familiar with the ASM1 terminology. In this review it has been chosen to focus on the methodologies, i.e. what can be achieved with different methods, their advantages and disadvantages, rather than focus on the different wastewater components and processes separately. This choice was motivated by the fact that some methods typically can yield information on more than one component or process. In the end of the review it is attempted to illustrate the power of the different methods for wastewater and sludge kinetics characterisation in the frame of ASM1. Finally, the relevance of characterising the different components and processes in the frame of ASM1 model calibration is critically evaluated.

4.1 WASTEWATER CHARACTERISATION

Wastewater can be characterised either with physical-chemical methods or with biological methods. In practice one typically ends up with a combined approach to obtain an estimate of the concentrations of all components. In the following physical-chemical and biological methods will first be described separately to obtain an overview of what can be achieved with the different methods. Finally, an overview of what can be achieved by combining both approaches is illustrated and discussed. In ASM1 the CODtot of the wastewater is considered to consist of inert soluble organic matter (S_I), readily and slowly biodegradable substrate (S_S and X_S respectively) and inert suspended organic matter (X_I), whereas biomass in the wastewater is considered to be insignificant:

$$\text{CODtot} = S_I + S_S + X_I + X_S \quad (5)$$

4.1.1 Physical-chemical characterisation

A wastewater can be separated into different components in a relatively simple manner via physical-chemical separation methods. The difference in molecular size can give an indication on biodegradability because small molecules can be taken up directly over the cell membranes whereas bigger molecules need to be broken down prior to uptake. Enzymatic hydrolysis is primarily a surface phenomenon, which means that the hydrolysis rate is directly related to the surface area. Thus, smaller molecules are readily degraded whereas degradation of larger material can be kinetically limited.

In early studies the wastewater components were separated physically into four size depending fractions by successive sedimentation, centrifugation, and filtration. The fractions were classified as settleable, supracolloidal, colloidal, and soluble (Rickert and Hunter, 1971), and were analysed for chemical oxygen demand (COD). An important conclusion from these studies was that the particles smaller than 1.0 μm were approximated to be the true soluble fraction. Moreover, the particles smaller than 1.0 μm were observed to be more rapidly degradable than particles larger than 1.0 μm. In a more recent study Levine *et al.* (1985) studied the size distribution of the organic matter in wastewater and the relationship to different wastewater treatment processes. In this study it was concluded that separation over a membrane with a pore size of 0.1 μm was valid for a differentiation between the true soluble and particulate organic fractions. The organic particles smaller than 0.1 μm are typically cell fragments, viruses, macromolecules and miscellaneous debris. The major groups of macromolecules in wastewater are polysaccharides, proteins, lipids and nucleic acids. The fraction measured by the standard test for suspended solids (1.2 μm) includes protozoa, algae, bacterial flocs and single cells. However some bacterial cells, cell fragments, viruses and inorganic particles have a size from 0.1 to 1.2 μm and will thus also pass through the more typically applied filter size of 0.45 μm for separation between soluble and particulate matter (Levine *et al.*, 1985). The size of colloidal matter is typically in the range 0.1-50 μm whereas material with a size larger than 50 μm usually settles (Levine *et al.*, 1985).

The ASM models do not differentiate between filtered, colloidal and settleable wastewater fractions. It is therefore necessary to convert the fractions resulting from a physical-chemical characterisation to the ASM components. The possibilities and limitations of physical-chemical methods to accomplish this task are summarised and discussed below.

4.1.1.1. Inert soluble organic matter S_I Soluble inert organic matter S_I is present in the influent, but, importantly, is also produced during the activated sludge process (Chudoba, 1985; Orhon *et al.*, 1989, Boero *et al.*, 1991; Germirli *et al.*, 1991; Sollfrank *et al.*, 1992). Most of the evidence for the production of soluble organics by micro-organisms is collected from experiments with simple known substrates, e.g. glucose (Chudoba, 1985; Boero *et al.*, 1991). However, the production has also been proven to take place with wastewater (Orhon *et al.*, 1989; Germirli *et al.*, 1991; Sollfrank *et al.*, 1992). The S_I production seems to depend on the initial substrate concentration and on cultivation conditions (Chudoba, 1985). A model has been proposed relating the S_I formation to the hydrolysis of non-viable cellular materials in the system, thereby linking the S_I production to the initial substrate concentration and the decay of the

produced biomass (Orhon *et al.*, 1989). This model was verified in a study with different industrial wastewaters and, although the data were not of very high quality, some evidence was given that the S_I production depends very much on the wastewater type (Germirli *et al.*, 1991). The hyphotesis that the S_I production originates from the decay process was, however, contradicted in a study on municipal wastewater (Sollfrank *et al.*, 1992) where it was concluded that the S_I production was related to the hydrolysis of slowly biodegradable COD of the incoming wastewater.

Thus, although the origin of the S_I production may remain unexplained, it seems clear that it does take place to various extents depending on different factors as mentioned above, resulting in a S_I concentration in the effluent that may be higher than the influent. Such S_I production is, however, not included in the ASM models, where S_I is considered a conservative component. To deal with this discrepancy between model concept and reality a simplified approach is typically applied by the definition of a fictive model influent concentration S_I which includes the produced S_I together with the real S_I influent concentration (Henze, 1992).

It is not possible to measure S_I directly and different approximations are therefore usually applied. Most often S_I is determined by the soluble effluent COD, which has appeared to be a good estimate for S_I in case of a low loaded activated sludge process (Ekama *et al.*, 1986). On the other hand Siegrist and Tschui (1992) suggested that the influent S_I could be estimated as 90% of the effluent COD. These approximations may hold in most cases, but a more correct approach would be to consider it as the soluble effluent COD minus the soluble effluent Biochemical Oxygen Demand (BOD) multiplied with a BOD/COD conversion factor (Henze, 1992). Furthermore, S_I can be determined as the soluble COD remaining after a long-term BOD test with the influent (Henze *et al.*, 1987; Lesouef *et al.*, 1992). The latter approach is in fact a combination of physical-chemical and biological methods. However, in case of significant S_I production during the test the influent S_I may be overestimated (Sollfrank *et al.*, 1992), which may lead to an underestimation of influent S_S eventually. Finally, a procedure was developed to distinguish between S_I of the influent wastewater and S_I produced during degradation (Germirli *et al.*, 1991). However, in order to achieve significant response glucose was added in these tests assuming that the wastewater under study resembled glucose, an assumption which may not hold in practice.

Summarising, it will be case depending whether it is needed to characterise the produced S_I or whether the model component can be approximated as described above.

4.1.1.2. Readily biodegradable substrate S_S The soluble COD fraction excluding the soluble inert organic matter (S_I) is mostly considered to be the readily biodegradable substrate S_S. The correctness of this approach does however evidently depend on the pore size of the filters used for the separation. As described above the "true" soluble fraction passes through a 0.1 μm filtration step according to Levine *et al.* (1985). However, in practice larger filter sizes are most often used, which may result in an overestimation of the soluble readily biodegradable substrate concentration, assuming that the definition of Levine *et al.* (1985) holds.

Another study confirmed that the fraction passing a 0.1 μm filter gave a good representation of the soluble readily biodegradable substrate (Torrijos *et al.*, 1994). It was confirmed biologically (via respirometry, see below for a detailed description) that

the studied wastewater did not contain any particulate readily biodegradable matter. In contrast with this, Spanjers and Vanrolleghem (1995) found, also via respirometry, that filtered wastewater (0.45 μm) had a lower biological response than unfiltered wastewater, indicating that parts of the readily biodegradable wastewater fraction was retained on the filter. Similarly, for an industrial wastewater it was found that the filtrate fraction produced via ultrafiltration (pore size < 0.001 μm) had a lower biodegradability (13% of CODtot) than the fraction determined with a respirometric characterisation method (20% of CODtot) (Bortone *et al.*, 1994). Further it was also found that part of the soluble COD can be slowly biodegradable (Sollfrank and Gujer, 1991).

Finally, a method based on flocculation with $Zn(OH)_2$ has been developed to remove colloidal matter of 0.1-10 μm that normally passes through 0.45 μm filter membranes, and was successfully applied to a phosphorus removal activated sludge system (Mamais *et al.*,1993). However, the flocculation has appeared to be rather sensitive to interference and appears highly depending on the pH value during the flocculation (Haider, 2000).

4.1.1.3. Inert suspended organic matter X_I The test proposed for the determination of S_I, as the residual soluble COD remaining after a long-term BOD test, by Lesouef *et al.* (1992) can also be applied to determine X_I. The X_I concentration is then determined as the residual particulate COD, assuming that X_I is not produced during the test. This assumption may, however, be questionable since X_I will be produced due to decay during the long-term BOD test and corrections for this will have to be considered.

4.1.1.4. Slowly biodegradable substrate X_S As mentioned earlier, a physical characterisation based on different molecular sizes can be used to distinguish between readily biodegradable substrate S_S and slowly biodegradable substrate X_S. In one study it has been proposed that X_S may be determined as the colloidal fraction defined by 0.1 – 50 μm (Torrijos *et al.*, 1994). However, this hypothesis could not be supported since the results indicated that the colloidals mainly disappeared according to a physical removal mechanism without any related biological oxidation. In another study of contact stabilisation, a multiple filtration procedure was used to isolate and monitor the variation in concentration of the colloidal fraction between 0.03 – 1.5 μm (Bunch and Griffin, 1987). Here it was further confirmed that colloidal matter was removed physically, probably by adsorption. However, the subsequent increase in soluble organic matter, and corresponding oxygen uptake resulting from breakdown of colloidal substrate, were not observed. Thus, based on these two studies it is not clear whether colloidals can be considered equal to X_S. Part of the colloidal substrate may be inert, as was probably the case in the example of Bunch and Griffin (1987), but this was not considered in these studies.

In addition, parts of the soluble substrate (Sollfrank and Gujer, 1991) and the settleable matters may belong to the X_S fraction making it rather problematic to characterise X_S entirely by a physical-chemical method.

Finally, if the components S_S, S_I and X_I are known and if it is assumed that the biomass concentration is negligible, X_S can be determined via a simple mass COD balance.

4.1.1.5. Biomass X_{BH} and X_{BA} It is not possible to distinguish biomass concentrations via a physical-chemical method.

4.1.1.6. Nitrogen components S_{NH}, S_{ND}, S_{NO}, X_{ND} The nitrogen components can rather easily be detected by physical–chemical analysis via a combination of standard analyses of ammonium, nitrite and nitrate and Kjeldahl nitrogen (TKN) on filtered and non-filtered samples (Henze *et al.*, 1987).

4.1.2 Summary and discussion of physical-chemical wastewater characterisation

Based on the descriptions and discussions above it can be concluded that a wastewater characterisation entirely based on physical-chemical characterisation alone will not be sufficient to obtain an accurate distribution of the organic substrate over the different ASM1 components (Fig. 6A). However, physical-chemical methods alone may be adequate for the estimation of the nitrogen components (Fig. 6B). In Fig. 6 the dashed line indicates the range of uncertainty with respect to the determination of the organic components.

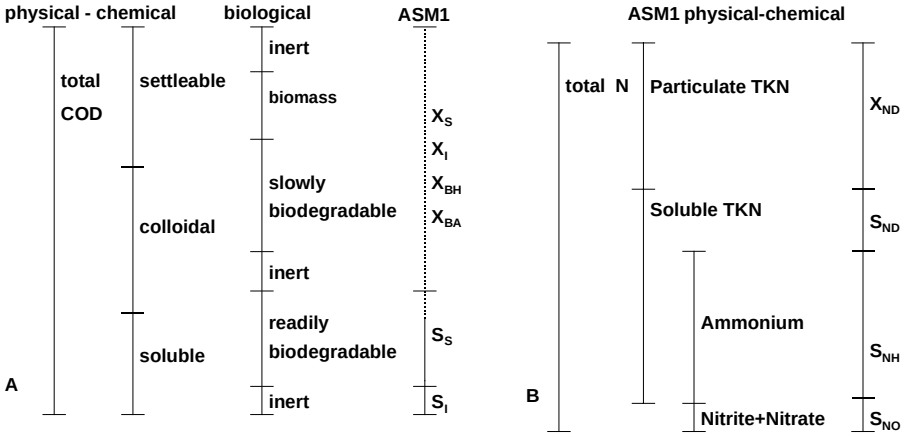

Fig 6. Characterisation of ASM1 wastewater components by physical-chemical methods (A: modified from STOWA, 1995; B: modified from Henze et al., 1995).

Summarising, the two main problems with respect to determination of the organic components entirely by physical-chemical means are:

- The reliability of S_S determination based on soluble COD depends very much on the applied filter size but, even more, on the kind of wastewater under study since it is possible that part of the particulate substrate is also readily biodegradable.
- Defining X_S as being the colloidals can induce errors because the colloidal fraction may also contain inert matter. Moreover, parts of the soluble and settleable fractions may belong to X_S. Thus, it is not possible to separate the particulate X_S, X_I and X_{BH} components adequately.

Table 5 summarises the characterisation of wastewater components via physical-chemical methods, and the assumptions needed, as described in the literature review above. According to this table it can be seen that with some assumptions and a combination of a physical-chemical and a biological method for assessment of X_I (long-term BOD test) (Lesouef *et al.*, 1992), it is possible to determine all COD components (S_S, S_I, X_S and X_I). Knowledge of X_I allows a determination of X_S via a mass balance of particulate COD, assuming that X_{BH} is zero. However, it should be kept in mind that the determination of X_I via a long-term BOD test may not be accurate, as discussed above. Moreover, the assumption that particular COD is not readily biodegradable may be incorrect.

Table 5. Overview of physical-chemical methods for determination of wastewater components (Fields with grey background indicate that a physical-chemical method is not applicable)

Component	Method	Additional information.	Assumptions	References
S_I	0.45 µm filtration of effluent		low loaded system (no biodegradable substrate in effluent)	E86
	90% of effluent COD			ST92
	7-8 µm filtration after long-term aeration test		no S_I production during degradation	L92; S92
	COD profiles in batch tests		wastewater similar to glucose	G91
S_S	0.1 µm filtration	S_I	Particulates do not contain readily biodegradable matters	L85; T94
	7-8 µm filtration	S_I		L92
	$Zn(OH)_2$ flocculation	S_I		M93
X_I	7-8 µm filtration after long-term aeration test		no X_I production during degradation	L92
X_S	mass balance	S_S, S_I, X_I	X_{BH}, X_{BA} negligible	H87
X_{BH}				
X_{BA}				
X_P				
S_O	Standard analysis of oxygen concentration			
S_{NO}	Standard analysis			H87
S_{NH}	Standard analysis			H87
S_{ND}	Standard analysis of soluble TKN	S_{NH}		H87
X_{ND}	Standard analysis of particulate TKN			H87
S_{ALK}	Standard analysis of alkalinity			

E86 Ekama *et al.*, 1986
G91 Germirli *et al.*, 1991
H87 Henze *et al.*, 1987
L92 Lesouef *et al.*, 1992
L85 Levine *et al.*, 1985
M93 Mamais *et al.*, 1993
ST92 Siegrist and Tschui, 1992
S92 Sollfrank *et al.*, 1992
S96 STOWA, 1996
T94 Torrijos *et al.*, 1994

4.1.3 Biological characterisation

The ASM models are in general biologically defined models. Thus, it is not surprising that biological wastewater characterisation methods have found wider application and acceptance than physical-chemical characterisation tests. In the biological methods the fractionation of organic matter is based on its rate of degradation (Henze, 1992) which makes the relation to the ASM concepts more direct. It is obvious that mainly the biodegradable components and the microbial biomass in the wastewater (S_S, X_S, S_{NH}, S_{ND}, X_{ND} and X_{BH}) can be characterised directly by these methods, whereas the inert components S_I and X_I may be determined by a combination of physical-chemical and biological tests, as already mentioned above (Lesouef *et al.*, 1992). Typically, a biological characterisation is based on measurements of the biomass response during substrate degradation in either a continuous flow-through system or batch type experiment. This means that the concentration determination of the biodegradable components is indirect, since the biomass activity has to be interpreted in terms of a substrate concentration. In principle the consumption of substrate can be measured directly by measurements of e.g. COD. However, this is typically not very practical due to problems of sampling and filtration of sludge samples etc. Instead, the biomass response can be monitored by recording the utilisation of electron acceptors (such as oxygen or nitrate), or the production of components during substrate degradation (such as protons, nitrate or carbon dioxide).

A main part of the review on biological characterisation will deal with respirometry. Respirometry is defined as the measurement and interpretation of the oxygen uptake rate of activated sludge (Spanjers *et al.*, 1998). In fact the main goal of a WWTP is to reduce the biochemical oxygen demand of the wastewater, and ASM1 was primarily developed to yield a good description of the sludge production and consumption patterns of electron acceptors, as described above. Thus, it is not surprisingly that respirometry has turned into one of the most popular biological characterisation methods, since the total respiration rate is affected by the concentration of all aerobically biodegradable components, to which the majority of wastewater components usually belong. However, nitrate utilisation rates can also be applied for characterisation of the denitrification potential of a wastewater. Finally, a titrimetric technique, especially applicable for determination of the ammonium concentration available for nitrification, will be reviewed.

Before the description and discussion on the application of respirometry, nitrate utilisation rates and titrimetry for wastewater characterisation, the methodology of each method is described in more detail. Thus, the readers already familiar with these methodologies can skip these intermediate sections and directly continue reading about their applications for wastewater characterisation.

4.1.3.1. Respirometry Historically, the determination of the Biochemical Oxygen Demand (BOD) during an incubation period of 5 to 7 days (BOD_5 or BOD_7) has been widely applied to quantify the effects of pollutants on the oxygen demand of receiving waters, and was further applied for the characterisation of wastewater. However, due to the rather arbitrary choice of 5 or 7 days the test result represents a varying part of the ultimate BOD of different wastewaters, depending on the wastewater composition. For a more complete analysis of the ultimate oxygen demand of a wastewater the BOD test

can be expanded to 20-30 days, typically 28 days. In the BOD tests the oxygen content is typically only recorded at the start and end of the test without information on the evolution of the oxygen consumption over time. This means that the test can not give any information on the different biodegradable fractions.

The test length of 5-7 days or even longer is not very suitable in the frame of wastewater treatment plant operation. As a consequence the concept of short-term biochemical oxygen demand (BOD_{st}) was introduced (Vernimmen *et al.*, 1967). The concentration of BOD_{st} can be determined via respirometry. As defined above, respirometry deals with the measurement and interpretation of the oxygen uptake rate, r_O, of activated sludge. In general, the r_O may be considered to consist of two components (Spanjers, 1993): The exogenous oxygen uptake rate ($r_{O,ex}$), which is the immediate oxygen uptake needed to degrade a substrate, and the endogenous oxygen uptake rate ($r_{O,end}$). Different definitions of $r_{O,end}$ appear in literature. The definition applied by Spanjers (1993) is that the $r_{O,end}$ is the oxygen uptake rate in absence of readily biodegradable substrate. In the context of ASM1 it is assumed that $r_{O,end}$ is associated with the oxidation of readily biodegradable matter produced by (1) hydrolysis of the slowly biodegradable matter that results from lysis of decayed biomass and, (2) the use of substrate for maintenance. The integral of the $r_{O,ex}$ profile is a measure of BOD_{st} (Spanjers *et al.*, 1998).

Contrary to the BOD_5 method, the BOD_{st} test is carried out with the same biomass as in the activated sludge plant under study and may therefore be a more representative measure of the effect of the wastewater on the particular activated sludge plant under study. Several attempts have been made to correlate BOD_5 to BOD_{st} (Vernimmen *et al.*, 1967; Farkas, 1981; Suschka and Ferreira, 1986; Vandebroek, 1986; Ciaccio, 1992; Vanrolleghem and Spanjers, 1994). However, the success of such a correlation seems to depend strongly on the type of wastewater, since the wastewater may contain varying proportions of readily and slowly biodegradable fractions.

Fig. 7 illustrates the conceptual idea of respirometry. The degradation of substrate S_1 and S_2 (Fig. 7A) results in a total exogenous uptake rate $r_{O,ex}$ (Fig. 7B). Fig. 7B illustrates a rather typical respirogram (i.e. a time course of respiration rates) with an initial peak in $r_{O,ex}$ caused by oxidation of the most readily biodegradable matter, here S_1, followed by, in this case, one "shoulder" in the $r_{O,ex}$ profile where component S_2 continues to be degraded. Thus, in this example the contribution of S_1 and S_2 to the total $r_{O,ex}$, and related total BOD_{st}, can easily be distinguished.

However, it will become clear from the "wheel-work" described in Table 6 (Vanrolleghem *et al.*, 1999) that most of the processes in ASM1 eventually act on the oxygen mass balance and may result in more complicated $r_{O,ex}$ profiles.

According to ASM1 the total $r_{O,ex}$ of the activated sludge in contact with wastewater is given in Eq. 6.

$$r_{O,ex} = (1 - Y_H) \cdot \frac{X_{BH} \cdot \mu_{max\,H}}{Y_H} \cdot \frac{S_S}{K_S + S_S} + (4.57 - Y_A) \cdot \frac{X_{BA} \cdot \mu_{max\,A}}{Y_A} \cdot \frac{S_{NH}}{K_{NH} + S_{NH}} \quad (6)$$

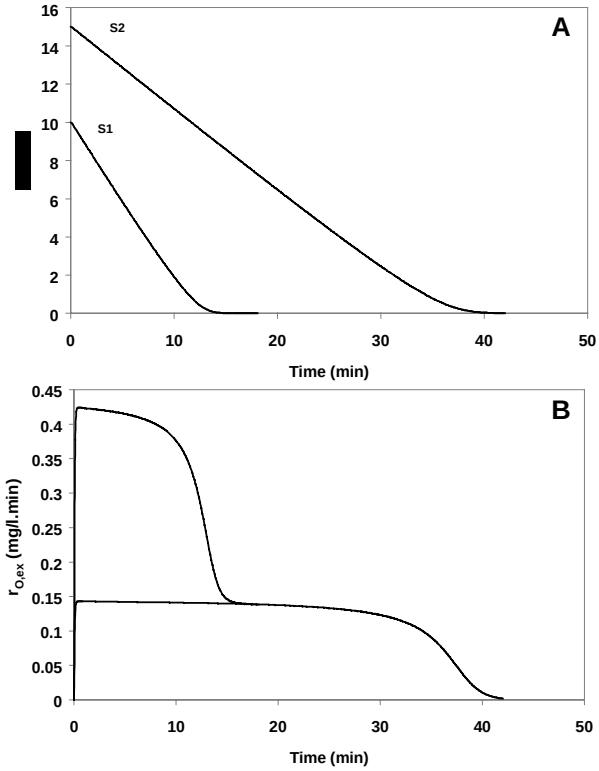

Fig 7. Conceptual respirogram resulting from degradation of substrate S_1 and S_2

The concentration of S_S and S_{NH} depend on the influent wastewater and also on the rates at which X_S, S_{ND} and X_{ND} are degraded. As an example we will follow the arrows from X_{BH} to S_O (Table 6): in the mass balance of the heterotrophic biomass X_{BH} (column, c., 5) the production of X_{BH} by aerobic growth (row, r., 1) is counteracted by the loss of X_{BH} by heterotrophic decay (r. 4). In this decay process component X_{BH} (c. 5) is converted to component X_S (c. 4). This production of X_S is counteracted by the loss of X_S by hydrolysis (r. 7), leading to production of component S_S (c. 2). S_S is subsequently used for heterotrophic growth (r. 1) where it is converted to component X_{BH} (c. 5) with concomitant consumption of oxygen S_O (c. 8), i.e. respiration. A similar reasoning can be made for the processes involving the nitrogen components (S_{NH}, S_{ND} and X_{ND}) and autotrophic (nitrifying) organisms (X_{BA}).

Fig. 8 shows different examples of respirograms collected in batch experiments where synthetic substrate (Fig. 8A) or different wastewaters (Fig. 8B-D) were added to endogenous sludge. Note that in Fig. 8C-D only the exogenous oxygen consumption due to substrate oxidation is given, $r_{O,ex}$, whereas the total r_O is given in Fig. 8A-B. It now becomes clear that the respirograms can differ significantly in shape depending on the substrate added and may not be as straightforward to interpret as the conceptual

example given in Fig. 7. Thus, the challenge is to interpret and perhaps divide the respirogram according to the contribution of $r_{O,ex}$ by different wastewater components.

There are two approaches for the determination of model parameters and components: *direct methods* focus on specific parameters and components which can directly be evaluated from the measured respiration rates (Ekama *et al.*, 1986; Spanjers *et al.*, 1999), whereas *optimisation methods* use a (more or less simplified) model that is fitted to the measured data (Kappeler and Gujer, 1992; Larrea *et al.*, 1992; Wanner *et al.*, 1992; Spanjers and Vanrolleghem, 1995; Brouwer *et al.*, 1998; Coen *et al.*, 1998). In the latter, numerical techniques are used to estimate parameter values that lead to the smallest deviation between model predicted and measured respiration rates (see Fig. 4).

Below, examples of respirometric experiments to assess the different wastewater components will be reviewed and important experimental factors with respect to wastewater characterisation will be discussed. The overview does not attempt to review and evaluate different respirometric principles, since a review of these is included in Spanjers *et al.* (1998) and Petersen (2000). Different methods may only be included here to illustrate points that are specifically related to wastewater characterisation.

Table 6. Kinetic and stoichiometric relationships for COD removal, nitrification and denitrification (Vanrolleghem et al., 1999) (cont' on next page)

	Component *i*	1	2	3	4	5	6	7	8
j	Process	S_I	S_S	X_I	X_S	X_{BH}	X_{BA}	X_P	S_O
1	Aerobic hetero-trophic growth		$-\frac{1}{Y_H}$			1			$1-\frac{1}{Y_H}$
2	Anoxic hetero-trophic growth		$-\frac{1}{Y_H}$			1			
3	Aerobic auto-trophic growth						1		$1-\frac{4.57}{Y_A}$
4	Het. decay				$1-f_P$	–1		f_P	
5	Aut. Decay				$1-f_P$		–1	f_P	
6	Ammonification								
7	Hydrolysis		1		–1				
8	Hydrolysis of N								
Observed conversion rates $ML^{-3}T^{-1}$		$r_i = \sum_j r_{ij} = \sum_j \nu_{ij}\rho_j$							
Stoichiometric parameters (see text)		Nomenclature, see text All units in ML^{-3} (COD or N, depending on variable)							

Readily biodegradable substrate S_s

The readily biodegradable substrate is presumably composed of simple and low molecular soluble compounds, such as volatile fatty acids, alcohols, etc. (Henze, 1992). The characteristic of these compounds is that they are degraded rapidly and hence result in a fast respirometric response, e.g. Fig. 8A.

The most typical batch test for determination of S_S involves the addition of a wastewater sample to endogenous sludge, and the monitoring of the respiration rate until it returns back to the endogenous level (Ekama *et al.*, 1986 among others). The examples shown in Fig. 8 are all obtained with such an approach. The respirometric methods may vary from a very simple lab-scale batch test to more complex methods that may even be applied on-line. The concentration of readily biodegradable substrate initially present in the mixture of biomass and wastewater in the experiment is generally calculated according to Eq. 7.

Table 6. Kinetic and stoichiometric relationships for COD removal, nitrification and denitrification (Vanrolleghem et al., 1999) (cont' from previous page)

9 S_{NO}	10 S_{NH}	11 S_{ND}	12 X_{ND}	13 S_{ALK}	Process rate ρ_j $ML^{-3}T^{-1}$
	$-i_{XB}$			$-\frac{i_{XB}}{14}$	$\mu_H \cdot X_{BH}$
$-\frac{1-Y_H}{2.86 \cdot Y_H}$	$-i_{XB}$			$\frac{1-Y_H}{2.86 \cdot 14 \cdot Y_H} - \frac{i_{XB}}{14}$	$\mu_H^O \cdot X_{BH}$
$\frac{1}{Y_A}$	$\frac{1}{Y_A} - i_{XB}$			$-\frac{2}{14 \cdot Y_A} - \frac{i_{XB}}{14}$	$\mu_A \cdot X_{BA}$
			$i_{XB} - f_P \cdot i_{XP}$		$b_H \cdot X_{BH}$
			$i_{XB} - f_P \cdot i_{XP}$		$b_A \cdot X_{BA}$
	1	-1		$\frac{1}{14}$	$k_a \cdot S_{ND} \cdot X_{BH}$
					$k'_h \cdot X_S$
		1	-1		$k_h \cdot X_{ND}$
$r_i = \sum_j r_{ij} = \sum_j \nu_{ij} \rho_j$					
Nomenclature, see text All units in ML^{-3} (COD or N, depending on variable)					Kinetic parameters (see text)

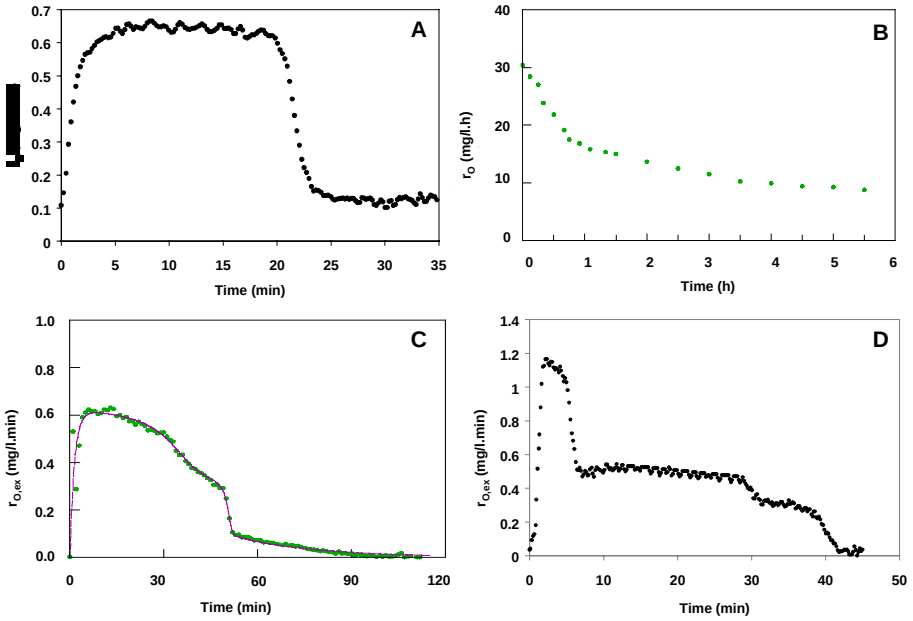

Fig 8. A: Typcial acetate profile B: Municipal wastewater (after Kappeler and Gujer, 1992), C: Municipal wastewater (Spanjers and Vanrolleghem, 1995), D: Industrial wastewater (Coen et al., 1998)

$$S_S(0) = \frac{1}{1 - Y_H} \cdot \left(\int_0^{t_{fin}} r_{O,ex}\, dt \right) \qquad (7)$$

The concentration of S_S in the wastewater is then easily calculated by taking the dilution into account. The end point t_{fin} of the integration interval is the time instant where S_S is completely oxidised and where the exogenous respiration rate for S_S becomes zero. The integral can directly and easily be obtained by determining the area under the $r_{O,ex}$ profile, e.g. by using a spreadsheet program. An alternative consists of solving the mass balance equations with a numerical integrator to predict the exogenous respiration rates for S_S and a given initial value $S_S(0)$. It may be a bit overdone to apply numerical integration for the profile illustrated in Fig. 8A, however for more complex profiles (Fig. 8B-D), the approach may become necessary and more straightforward than direct calculation, as will be discussed further below.

Notice that knowledge of the heterotrophic yield coefficient Y_H is needed for the calculation of S_S from respiration rates (Eq. 7). The yield indicates the COD fraction that is converted to cell mass. The rest of the COD is used to provide the energy that is required to drive different synthesis reactions. This energy is made available by

oxidative phosphorylation, which requires a terminal electron acceptor, in this case oxygen. The produced energy is proportional to the mass of electron acceptor utilised, which in turn is proportional to the COD consumed. As a consequence $(1-Y_H)\cdot$COD is equal to the integral under the $r_{O,ex}$ curve. Evidently, the parameter Y_H is always involved when oxygen consumption is converted to substrate equivalents.

The batch test described above is also used to assess other ASM1 components and, likewise, kinetic and stoichiometric parameters. This will be explained further in the next section on characterisation of sludge kinetics, but this indicates already the popularity of this test in assessing wastewater components and reaction kinetics.

Apart from the typical batch test as described above, other experimental designs have also been tried out for the determination of S_S. One example consists of monitoring the respiration rate of unsettled sewage without inoculum for a relatively long period, approximately 20 hours (Wentzel *et al.*, 1995). A respirogram similar to the one depicted in Fig. 9 is obtained. The S_S concentration is calculated from the respiration rates observed between the start of the test up to the time with the precipitous drop (due to depletion of S_S), with correction for the increasing endogenous respiration due to the increase of biomass during the test. In addition to Y_H, knowledge of the maximum specific growth rate is required, information that can be obtained from the same test (see below).

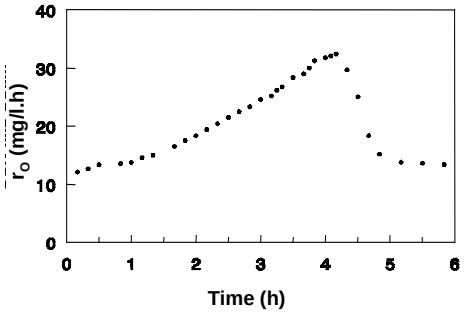

Fig 9. Respiration rates measured in a batch experiment for estimation of μ_{maxH} and K_S (after Kappeler and Gujer, 1992.

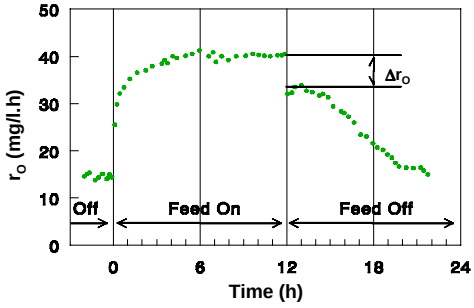

Fig 10. Respiration rates obtained with the experimental set-up of Ekama et al. (1986)

An often-referred continuous flow-through method was developed by Ekama *et al.* (1986), see Fig. 10. This method involves the monitoring of respiration rate in a completely mixed reactor operated under a daily cyclic square-wave feeding pattern. The experiment is designed in such a way that the supply of S_S from hydrolysis of X_S remains constant for a period after the feed is stopped and gives rise to a second r_O plateau. It is hypothesised that the difference in r_O plateau values corresponds uniquely to the S_S that has entered via the influent. Hence, the concentration of readily biodegradable substrate in the wastewater can be calculated as given in Eq. 8.

$$S_S = \frac{V}{Q} \cdot \frac{\Delta r_O}{(1 - Y_H)} \quad (8)$$

An obvious disadvantage of this method is the length of the experiment (24 h, which is not including the stabilisation of the continuous reactor used for the test), and the fact that sufficient X_S is needed in the feed to achieve a constant hydrolysis rate and to create as such the step change in r_O. In addition, the method is rather difficult to carry out in practice (Sollfrank and Gujer, 1991; Wentzel *et al.*, 1995).

A final method for the evaluation of S_S was based on the evolution of the respiration rates obtained in a continuously fed respirometer during transients between two modes of operation; a mode of endogenous respiration and wastewater addition respectively (Spanjers *et al.*, 1994). In the work of Lukasse *et al.* (1997) the estimation technique developed for the determination of S_S in the respirometer of Spanjers *et al.* (1994) was further evaluated and improved. In the work of Witteborg *et al.* (1996) the same continuously fed respirometer was used but a different estimation of S_S was proposed as now the measurement of respiration rate was performed under three different wastewater loading conditions. The wastewater S_S was calculated by numerically solving a set of mass balances pertaining to different loading conditions of the respirometer.

Slowly biodegradable substrate X_S

It is assumed that slowly biodegradable substrate X_S is composed of (high-molecular) compounds ranging from soluble to colloidal and particulate (Henze, 1992). The common feature of these compounds is that they cannot pass the cell membrane as such, but have to undergo hydrolysis to low-molecular compounds (S_S) which are subsequently assimilated and oxidised. The respirometric response on X_S is slower because the hydrolysis rate is lower than the oxidation rate of S_S.

In a batch test an exponentially decreasing "tail" can frequently be observed in respirograms (Fig. 8B-C). In Fig. 8B, this tailing starts after approximately 0.75 hours. The wastewater concentration of X_S can be assessed in a similar way as above, Eq. 7 (Sollfrank and Gujer, 1991; Kappeler and Gujer, 1992). Simultaneously occurring oxidation processes such as nitrification might interfere and complicate the separation of the respiration rate due to hydrolysis in the total respiration rate. In that case a nitrification inhibitor may be used to facilitate the assessment of X_S (Spanjers and Vanrolleghem, 1995). Alternatively, if the data of such respirometric batch tests are used in combination with mathematical curve fitting techniques to match the response of the model to the data, the nitrification part can rather easily be extracted from the respirogram (Spanjers and Vanrolleghem, 1995).

It has also been proposed to estimate X_S based on a long-term BOD test where X_S is obtained by subtracting S_S from BOD/(1-Y_H) (STOWA, 1996). Note that the value of Y_H here should be lower than the one applied in Eq. 7, due to internal turnover of substrate from decayed biomass in long-term tests.

Heterotrophic biomass X_{BH}

In the ASM1 report the influent concentration of heterotrophic biomass, X_{BH}, is assumed to be negligible, as mentioned earlier. However, some wastewaters can contain

a significant concentration of heterotrophic biomass (Henze, 1992), and there may therefore be a need to quantify this component. A batch test has been proposed where X_{BH} is assessed from the respirometric response of raw wastewater without inoculum (Kappeler and Gujer, 1992; Wentzel *et al.*, 1995). The calculation requires knowledge of Y_H together with two parameters (μ_{maxH} and b_H) that can be obtained from the same data. Respirograms look similar to the one presented in Fig. 9. The procedure basically backtracks the amount of heterotrophic biomass originally present in the wastewater by comparing the original respiration rate with the respiration rate after significant (hence, well quantifiable) growth of X_{BH}.

Autotrophic biomass X_{BA}

So far, no procedures were found by which the autotrophic biomass concentration in wastewater is determined. However, it could be imagined that a similar procedure as the one developed for X_{BH} is applicable. Thus, by evaluation of the respiration rate for nitrification, $r_{O,ex}^{N}$, of the autotrophs present in the wastewater and by comparison to the respiration rate of a culture with known autotrophic biomass concentration X_{BA}, e.g. after significant growth, the originally present X_{BA} could be determined.

Ammonium S_{NH}

The concentration of ammonium in wastewater can be determined by using conventional analytical techniques, as mentioned earlier. However, respirometry also offers the possibility to deduce S_{NH} from batch measurements in a similar way as S_S and X_S, provided the test is done with nitrifying activated sludge and the oxygen consumption for nitrification can be separated from the other oxygen consuming processes. As follows from Table 6, the autotrophic yield coefficient Y_A is needed to convert the oxygen consumption for nitrification to a nitrogen concentration by division by $(4.57\text{-}Y_A)$, where 4.57 indicates the amount of oxygen needed to oxidise one unit of ammonium nitrogen. The value of Y_A is typically 0.24 g COD(biomass)/g N, which means that the determination of S_{NH} is not very sensitivity to Y_A since its value is small compared to 4.57.

Notice that part of the available ammonium may be assimilated into new heterotrophic biomass, which may be a considerable fraction of the nitrogen in case a large amount of COD is biodegraded ($COD^{Degraded}$) simultaneously with the nitrification. The actual nitrified ammonium nitrogen, denoted N^{Nitr}, can be approximated by Eq. 9 in which i_{XB} is the nitrogen content of newly formed biomass:

$$N^{Nitr} = S_{NH} - i_{XB} \cdot Y_H \cdot COD^{Degraded} \qquad (9)$$

From this equation one can easily deduce the original nitrogen concentration when $COD^{Degraded}$, and the stoichiometric parameters i_{XB} and Y_H are given. Note, however that fitting a model in which carbon and nitrogen oxidation are included to the respirometric data will automatically take this correction into account (Vanrolleghem and Verstraete, 1993; Spanjers and Vanrolleghem, 1995; Brouwer *et al.*, 1998).

Organic nitrogen S_{ND} and slowly biodegradable organic nitrogen X_{ND}
Probably because the ammonification and hydrolysis rates of organic nitrogen compounds are relatively fast, little attention has been devoted so far to the establishment of respirometric techniques for S_{ND} and X_{ND} quantification. In batch tests these compounds are typically converted to S_{NH} before the S_{NH} that was originally present in the wastewater is removed by nitrification. Therefore, S_{ND} and X_{ND} are not directly observable in such tests but may be lumped into the fraction of nitrified ammonium. Still, for some industrial wastewaters the ammonification and hydrolysis steps may be considerably slower and quantification of these component concentrations may be required. In such cases, one can imagine a procedure in which the nitrification respiration rate $r^{N}_{O,ex}$ is monitored and interpreted in terms of ammonification and hydrolysis, similar to the way the respiration resulting from COD degradation is interpreted in terms of the biodegradation of readily biodegradable substrate and the hydrolysis process. Subsequently, the amounts of nitrogen containing substrates could be assessed by taking the integral of $r^{N}_{O,ex}$ for the corresponding fractions and dividing these by $(4.57\text{-}Y_A)$. In case simultaneous COD-removal is taking place, correction should again be made for nitrogen assimilated into new heterotrophic biomass (see above).

4.1.3.2. Nitrate utilisation rates

Readily or slowly biodegradable substrate S_S and X_S
The basis for wastewater characterisation via monitoring of nitrate utilisation rates (r_{NO3}) for the determination of the denitrification potential is rather similar to that of respirometry (Nichols *et al.*, 1985*;* Ekama *et al.*, 1986; Kristensen *et al.*, 1992; Naidoo *et al.*, 1998; Spérandio, 1998; Urbain *et al.*, 1998; Kujawa and Klapwijk, 1999). The application of nitrate utilisation rates for wastewater characterisation within the frame of ASM1 is however not as widespread as respirometry.

The readily biodegradable component S_S (or X_S) is determined by Eq. 10 (similar to Eq. 7). A typical r_{NO3} profile is given in Fig. 11, indicating two biodegradable wastewater fractions.

$$S_S(0) = \frac{2.86}{1-Y_H} \cdot \left(\int_0^{t_{fin}} r_{NO3,ex}\, dt \right) \qquad (10)$$

The factor 2.86 gO_2/gNO_3-N originates from the fact that the theoretical electron acceptor capacity of nitrate (as N) is 2.86 times that of oxygen (as O), assuming that NO_3-N is converted completely to nitrogen gas N_2 (Payne, 1981; van Haandel *et al.*, 1981). The factor has been verified experimentally by Copp and Dold (1998).

In Eq. 10 it is assumed that the Y_H of aerobic and anoxic substrate degradation is equal, as also assumed in ASM1. In a study on a pure denitrifying culture it has however been reported since long that aerobic yields are larger than anoxic yields (Koike and Hattori, 1975). It has been theoretically proven, based on the energetics of

the metabolic processes, that anoxic yields indeed are consistently lower than aerobic ones (Orhon *et al.*, 1996). Indeed similar differences between aerobic and anoxic yield were obtained experimentally with activated sludge (McClintock *et al.*, 1998; Spérandio *et al.*, 1999). Thus, to apply nitrate utilisation rates for wastewater characterisation it is important to correct for this difference in aerobic and anoxic yield since application of aerobic yield values in Eq. 10 will lead to overestimation of the readily biodegradable wastewater components.

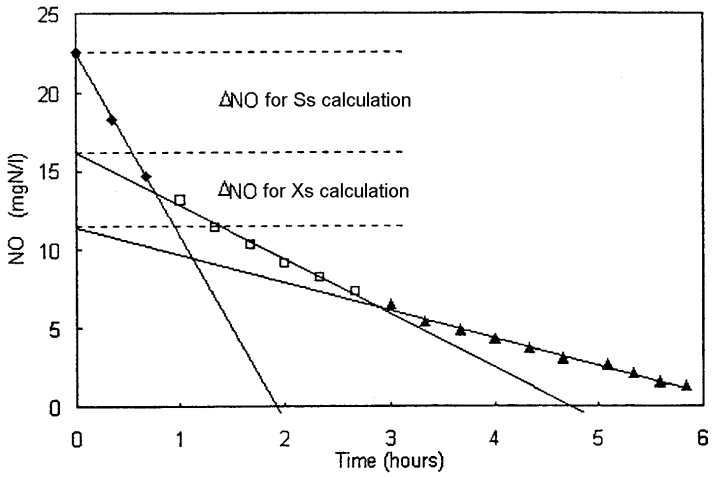

Fig 11. Typical profile of r_{NO3} as function of time for determination of S_S and X_S (Urbain et al., 1998)

4.1.3.3. Titrimetry The buffer capacity of water samples can be measured accurately by advanced titration techniques (Van Vooren *et al.*, 1995), and has recently been successfully applied for the determination of ammonium and phosphorus in low concentrations (0 – 100 mg/l) in effluents, surface waters and manure (Van Vooren, 2000).

Some efforts have been done to characterise VFA concentrations related to anaerobic processes based on titration procedures and pH measurements (e.g. Münch and Greenfield, 1998). These techniques may also be applicable for wastewater characterisation in the frame of ASM2 where one component is defined as the concentration of fermentation products. This will however not be dealt with any further in this presentation.

Alternative to the classical titration methods (up and down titrations) Ramadori *et al.* (1980) proposed to monitor the acid and/or base consumption rate that was needed to keep the pH constant in an activated sludge sample where pH-affecting biological reactions occur. This titrimetric method has been successfully applied for the monitoring of nitrification, which has a clearly defined effect on the pH, and concentrations of S_{NH} (Massone *et al.*, 1995; Gernaey *et al.*, 1997). Recently, it has also

been attempted to apply the method for the determination of the total nitrifiable nitrogen concentration of a wastewater (Yuan *et al.*, 1999).

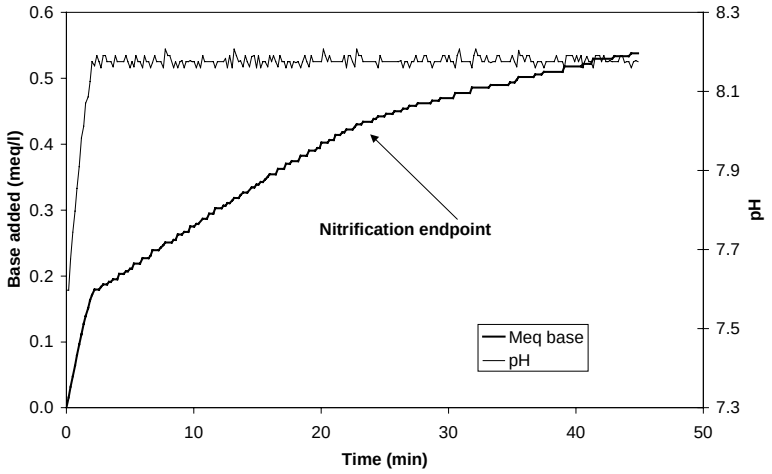

Fig 12. Typical cumulative base addition curve (expressed as amount of base dosed per liter of activated sludge sample) and pH profile obtained during an on-line titration experiment with a mixed liquor sample. For this example, the nitrification phase is finished after about 25 minutes (Gernaey et al., 1998).

Ammonium, S_{NH}

A typical cumulative base addition curve and a pH profile collected during a titration experiment with nitrifying sludge sampled on-line from a pilot plant are shown in Fig. 12 (Gernaey *et al.*, 1998). In a first phase, the pH of the sludge sample is increased to the pH setpoint, and base is added at a maximum rate. This phase took about 2 minutes for the example of Fig. 12 For the experiments described here, a pH setpoint ± ΔpH interval value of 8.2 ± 0.03 was used. Every time the pH of the sludge sample becomes lower than 8.17 (= pH setpoint minus ΔpH interval), base is added to the sludge. Dosage of base is repeated until the pH has returned within the pH setpoint ± ΔpH interval range. Here, the nitrification phase is finished after about 25 minutes.

The analysis of the data can either be via a simple manual interpretation or model-based (Gernaey *et al.*, 1998). The simple procedure is based on the detection of the two slopes (S1 and S2) in the cumulative base addition curve, followed by an extrapolation of the different lines to the Y-axis (Fig. 13). The S_{NH} concentration (mg N/l) and the nitrification rate r_N (mg N/l.min) can be calculated according to Eq. 11 and 12, where the intercepts B1 and B2 are expressed in meq/l units. The factor 0.143 meq/mg N (i.e., 2 mole H^+ per mole N), is the stoichiometric coefficient relating the amount of acid (meq) produced per mg of nitrogen nitrified. The slopes S1 and S2 are expressed in meq/l.min units.

$$S_{NH} = \frac{(B2 - B1)}{0.143} \quad (11)$$

$$r_N = \frac{(S1 - S2)}{0.143} \quad (12)$$

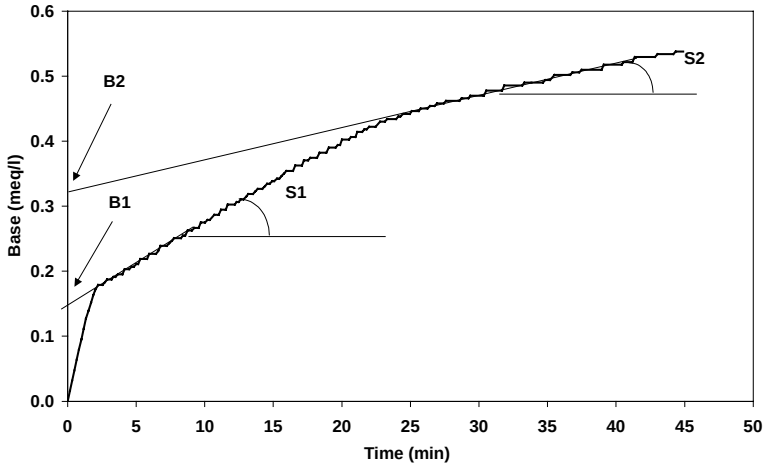

Fig 13. Simple manual interpretation of a typical cumulative base addition curve (Gernaey et al.*, 1998).*

In the application of Gernaey *et al.* (1998) the sludge was sampled at the last compartment of an activated sludge pilot plant thereby reducing the likelihood of presence of organic substrates. In case ammonification is slower than nitrification it may be relevant to determine S_{ND}, as described above in the section on respirometry. Thus, the titrimetric method may also be applicable for S_{ND} determination. It may be foreseen, however, that degradation of organic substrates may cause acid or base consumption effects that may interfere with the determination of S_{NH} according to the described methodology.

Readily biodegradable substrate S_S.

The titrimetric methodology has also been applied for the determination of readily biodegradable COD available for denitrification, and within control strategies for additional carbon dosage (Bogaert *et al.*, 1997). A complicating factor is that depending on the carbon source denitrification will either produce or consume acid (Bogaert *et al.*, 1997). Preliminary results (Dhaene, 1996; Rozzi *et al.*, 1997) have indicated that the method may be used to evaluate S_S in concentrated wastewaters.

4.1.4 Summary and discussion of biological wastewater characterisation

The capabilities of the different biological methods presented above to directly determine the ASM1 wastewater components are illustrated in Fig. 14 (the dashed lines

indicate areas of uncertainties) and summarised in Table 7. According to Fig. 14 it is obvious that the readily biodegradable organic wastewater components, i.e. S_S and parts of X_S (Fig. 14A), and the nitrogen components S_{NH} and parts of S_{ND} and X_{ND} (Fig. 14B), can be determined directly via the biological methods. The determination of the slower biodegradable component X_S can be carried out indirectly via a long-term BOD test and knowledge of S_S (STOWA, 1996). However, uncertainties may be introduced by long-term BOD tests since significant interference from product formation may occur during the lengthy test.

Table 7. Overview of biological methods to estimate wastewater component concentrations. (Fields with grey background indicate that a respirometric method is either not applicable or not relevant. For an explanation of the references, see Table 8)

Component	Method	Type of experiment	*Additional* Information	Assumptions	References
S_I	R	BOD_∞, WW	Y_H		H87; L92
S_S	R	B, WW add. B, WW C C (on/off)	Y_H Y_H, μ_{mH}, K_S Y_H Y_H		E86 We95 Wi96 E86; SG91; We95
	N	B, WW add.	Y_H		E86; K92; N98; U98; KK99
	T	B, WW, S	C/N	C/N constant	B97; R97; D96
X_I					
X_S	R	B, WW BOD_∞, WW	Y_H		SG91;KG92; SV95 S96
		B, WW add.	Y_H		N98; U98; KK99
X_{BH}	R	B, WW	Y_H		KG92; We95; B95
X_{BA}	R	B, WW	Y_A		This paper
X_P					
S_O					
S_{NO}	T	B, S	C/N	C/N constant	B97
S_{NH}	R	B, WW	Y_A, i_{XB}, Y_H, COD^{Deg}		VV93, SV95; Br98
	T	B, WW			M95, G97; G98
S_{ND}	R	B, WW	Y_A, i_{XB}, Y_H, COD^{Deg}		This paper
X_{ND}	R	B, WW	Y_A, i_{XB}, Y_H COD^{Deg}		This paper
S_{ALK}					

For the determination of S_{NH} it should be remembered that it is in fact the nitrifiable nitrogen that is determined via the biological methods (as indicated with dashed lines into the regions of organic nitrogen, since parts of the organic nitrogen may be hydrolysed making it readily available for nitrification). This is in contrast to the physical-chemical method where the S_{NH} component is determined via a chemical analysis of ammonia.

4.1.5 Discussion on physical-chemical vs. biological wastewater characterisation

By definition the total COD in ASM1 is sub-divided based on (1) solubility, (2) biodegradability, (3) biodegradation rate and (4) viability (biomass), as described earlier. Summarising, the COD components to consider in a wastewater are:

$$CODtot = S_I + S_S + X_I + X_S + (X_{BH}) \quad (13)$$

In previous sections it has been thoroughly reviewed how to determine these components by either physical-chemical or biological methods, and different limitations of the methodologies have been underlined and discussed. Furthermore, it is obvious that the division of the wastewater into model components is to some extent artificial. For example, a division is made between soluble and readily biodegradable substrate (S_S) and particulate slowly biodegradable matter (X_S), although it is, for example, known that some slowly biodegradable substrate may be soluble etc.

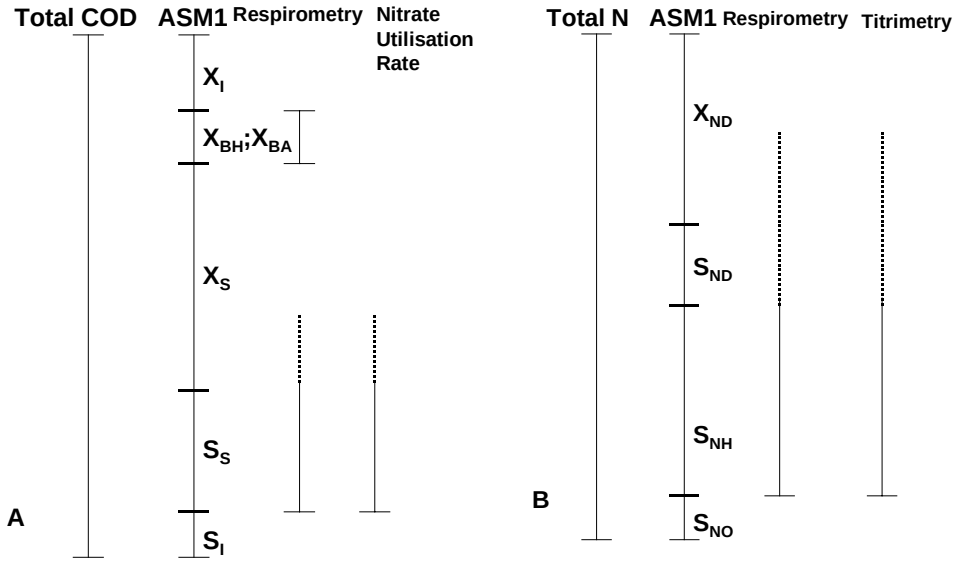

Fig 14. Characterisation of ASM1 wastewater components by different biological methods (the dashed lines indicate areas of uncertainties). A: COD components; B: Nitrogen components

It became clear that an application of physical-chemical methods alone is not sufficient for characterisation of the wastewater into model COD components. These methods basically only allow to distinguish between soluble and particulate COD and do not differentiate with respect to biodegradability (non-biodegradable versus biodegradable matters) and biodegradation rate (readily versus slowly biodegradable substrates). However, by application of biological characterisation methods it is possible to obtain knowledge of the biodegradability and biodegradation rate of the wastewater.

Thus, it is obvious that a combination of physical-chemical and biological characterisation methods is advantageous for the translation of the wastewater characteristics into the ASM1 model components. A suggestion for such a combined approach, based on the literature review above, is presented in Fig. 15. Here it is suggested to determine the readily biodegradable substrate (S_S) directly via respiration

tests (respirometry or nitrate utilisation rates). The presence of biomass in the wastewater may also be determined by respiration tests. The slowly biodegradable matter (X_S) can be determined via the results of a long-term BOD test. The same kind of test may provide information on the soluble and particulate inert (S_I and X_I) matters. Here, however, the reservation should be repeated that long-term BOD tests may not be very accurate due to possible product formation (S_I) and decay which results in X_I. Therefore, the determination of X_I via a long-term BOD test may be questionable. Indeed, it is proposed by Henze *et al.* (1987) to determine the influent X_I via the complete model during the calibration of the sludge balance. Subsequently, X_S may be determined via a COD mass balance as the difference between total COD and the other components. If it is chosen to determine S_I by a long-term BOD test, it may be advisable to combine it with analyses of the effluent, as proposed in the section about physical-chemical methods. It is again clear from Fig. 15 that the borderline especially between particulate and soluble COD, the differentiation between model components (S_S and X_S) and the results from short-term respiration and long-term BOD tests may not be completely consistent.

The nitrogen ASM1 components are somewhat easier to determine since they can basically all be determined via mass balances based on standard chemical analyses of total nitrogen, Kjeldahl nitrogen, ammonium nitrogen and nitrate nitrogen (see Fig. 6). It can, however, be advantageous to combine these chemical analyses with biological methods (respirometry or titrimetry) to obtain the nitrifiable nitrogen as a measure of S_{NH} (see Fig. 14) for studies where the focus is specifically on nitrification capacities.

In a study of STOWA (STOWA, 1996) a similar, but less extensive, study of physical-chemical versus biological (only respirometric) influent wastewater characterisation was carried out. In this study guidelines for the COD components were finally defined based on a more traditional choice of physical-chemical methods combined with long-term BOD measurements to allow for an easy implementation in already existing routine analysis programs. It was concluded that respirometry is not yet at a state where it can easily be applied for routine wastewater characterisation. The STOWA guidelines for determination of the COD components are summarised in Fig. 16. Here the concentration of inert soluble matters (S_I) is determined as 90% of the effluent COD for low loaded systems, according to Siegrist and Tschui (1992). For high loaded systems S_I is also determined as 90% of the effluent COD but the effluent BOD (multiplied by a COD/BOD factor) is subtracted. S_S is determined as the difference between soluble COD and S_I. Furthermore, the concentration of X_S is based on a long-term BOD test as the difference between BOD/(1-Y_H) and S_S, as described above. The yield coefficient in this long-term test is set to 0.20. Finally, X_I is defined as the difference between particulate COD and the determined X_S. Obviously, in this approach the division of the wastewater into ASM1 components is based on solubility and to some extent on biodegradability according to physical-chemical methods supplemented by measurements of the ultimate BOD_∞ or BOD_5. The problem with this approach is that the biodegradation rate of the wastewater is not really considered. This means that the division of the biodegradable substrate into readily and slowly biodegradable substrates may not be correct. It should be stressed though that the approach chosen by STOWA is simple to implement into existing standard measuring routines at full-scale WWTP's, which is a factor not to be underestimated.

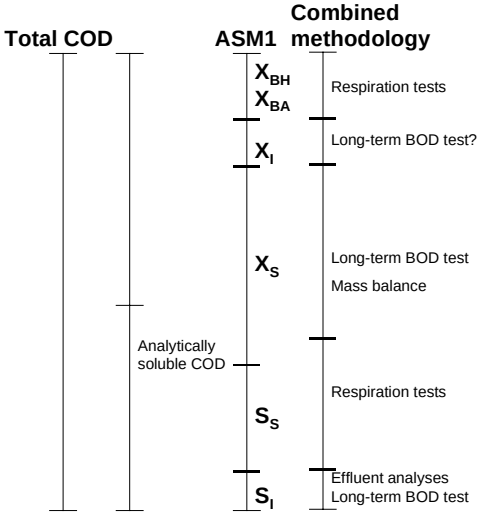

Fig 15. Suggested wastewater characterisation by combined physical-chemical and biological methods

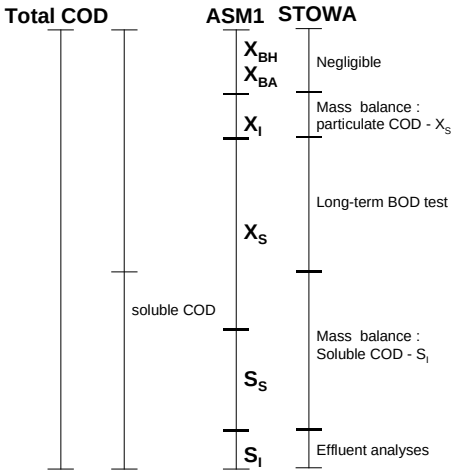

Fig 16. The STOWA (1996) guidelines for determination of COD components

The STOWA guidelines for nitrogen components are also rather simple and based on physical-chemical analyses. The S_{NH} component is obtained based on standard analyses of soluble ammonium nitrogen, and the determination of the organic nitrogen fractions (S_{ND} and X_{ND}) is based on certain fixed fractions of N in organic components. It is advised that these organic nitrogen fractions are checked regulary based on measurements of total nitrogen, Kjeldahl nitrogen etc. according to Fig. 6B.

In this literature review the focus has been on characterisation of the ASM1 wastewater components. However, with the introduction of ASM3 (see Table 2), that also focuses on a description of oxygen consumption, sludge production and N removal, it is interesting to discuss whether the approaches for wastewater characterisation applied for ASM1 holds for ASM3 as well.

As described above, there is a shift of emphasis from hydrolysis to storage of organic matter in ASM3. Furthermore, all S_S is supposed to go through the storage process (conversion to X_{STO}) before being used for growth. This means a change in how wastewater characterisation should be viewed, since the separation between S_S and X_S should now be based on the storage process rather than on the growth process. In ASM3 (Gujer *et al.*, 1999) it is supposed that the soluble (S_S) and particulate (X_S) biodegradable components can be differentiated with filtration over 0.45 μm membrane filters, whereas a significant fraction of X_S in ASM1 may be contained in the filtrate of the influent wastewater. In ASM3 the latter is assumed to be caused by the conversion of soluble biodegradable COD to storage polymers in the respiration tests. Whether this may hold in any case seems yet rather unclear. In Gujer *et al.* (1999) it was recognised

that the model concept of converting all S_S into a storage component is not in accordance with reality. Indeed, it was illustrated by Krishna and van Loosdrecht (1999) that the difference between feast and famine phases could not be described accurately. This was caused by the fact that ASM3 does not allow growth on the substrate S_S alone. Therefore, a new model structure was proposed where growth on external substrate is allowed in parallel with the storage process. It remains however uncertain how to differentiate between the amount of S_S that is directed to storage and growth respectively. Furthermore, the yield coefficient (which is needed to convert respirometric responses to COD components) in ASM3 is composed of two factors: $Y_{net}=Y_{STO}\cdot Y_H$, where Y_{STO} is the storage yield and Y_H the heterotrophic yield for the growth process. Also, here it does not seem clear how to differentiate between the two yields. Basically, concerning the characterisation of COD wastewater components, more experience will be needed before a wastewater characterisation of the COD components related to the new storage concept of ASM3 can be proposed.

The characterisation of the nitrogen components in ASM3 is however simplified by the fact that organic nitrogen components are included in the model as a fraction of the corresponding COD components. Degradation of the corresponding COD component results in immediate release of the organic nitrogen as ammonium. The latter was based on the assumption that the ammonification is fast and the conversion of organic nitrogen into ammonium therefore hardly affects the model predictions (Gujer *et al.*, 1999). Thus, the nitrogen balance includes on the one hand ammonium nitrogen (S_{NH}) and nitrate nitrogen (S_{NO}), which both can be measured easily via standard chemical analyses, and on the other hand organic nitrogen components. However, typically the fractions of organic nitrogen in the COD components can be considered to be constant.

4.2 CHARACTERISATION OF SLUDGE COMPOSITION

In this section special attention is only paid to the assessment of the slower varying sludge characteristics. Knowing the initial value of the concentrations of soluble components (e.g. ammonia) is not really essential because it has little impact on typical simulation results with a calibrated model. Hence, the concentrations of the following particulate, slowly varying components must be assessed: X_{BH}, X_{BA} and X_I ($+X_P$), assuming that the system is in balance with no accumulation of X_S. Only two concentrations must be assessed since the sum of the concentrations is equal to the particulate COD (X) of the sludge that can easily be measured by using traditional COD analysis (Eq. 14)

$$X = X_I + (X_P) + X_{BH} + X_{BA} \qquad (14)$$

Below some fast and direct methods for assessing sludge components are summarised. Notice that the particulate nitrogen components are not considered here as their concentrations are assumed to be low.

Heterotrophic biomass X_{BH}

One can show that the concentration of heterotrophs in a continuous system in steady state is equal to:

$$X_{BH} = Y_H \cdot \frac{\theta_X}{\theta_H} \cdot \frac{COD^{Degraded}}{1 + b_H \cdot \theta_X} \quad (15)$$

where θ_X is the sludge age, θ_H is the hydraulic retention time, $COD^{Degraded}$ the total amount of COD removed (taken over a sufficiently long period, e.g. one sludge age), b_H the decay rate coefficient and Y_H the yield coefficient. Respirometric methods to determine the parameters b_H and Y_H are discussed below, while a respirometric evaluation of $COD^{Degraded}$ can be performed with the respirometric measurements of biodegradable COD fractions (S_S, X_S) that was already presented above.

As an alternative, Bjerre *et al.* (1995) used the method of Kappeler and Gujer (1992) to determine the concentration of heterotrophs in the mixed liquor. Recently, this method was thoroughly evaluated by Ubisi *et al.* (1997).

Autotrophic organisms X_{BA}

In much the same way, the concentration of nitrifying organisms in the activated sludge can be evaluated by means of a mass balance for the autotrophs (over a sufficiently long time) (Dupont and Sinkjær, 1994):

$$X_{BA} = Y_A \cdot \frac{\theta_X}{\theta_H} \cdot \frac{f^{Aerobic} \cdot N^{Nitr}}{1 + b_A \cdot \theta_X} \quad (16)$$

where $f^{Aerobic}$ is the aerobic fraction of the reactor; N^{Nitr} the amount of nitrified nitrogen ; b_A the autotrophic decay rate coefficient and Y_A the autotrophic yield coefficient. The methods to determine the parameters b_A and Y_A are discussed in the next paragraph, while N^{nitr} can be quantified using the respirometry-based nitrifiable nitrogen evaluation methods that were given above.

Produced inert suspended organic matter X_P

To determine the produced inert matters, X_P, an evaluation of the mass balance of X_P in steady state can be made. Assuming that the autotrophic biomass can be neglected, Eq. 17 is obtained:

$$X_P = f_P \cdot b_H \cdot X_{BH} \cdot \theta_X \quad (17)$$

The total concentration of inert matters, including the often significant contribution of suspended inerts from the influent, is given in Eq. 18.

$$X_P = \frac{\theta_X}{\theta_H} X_i + f_P \cdot b_H \cdot X_{BH} \cdot \theta_X \quad (18)$$

Respirometry can be involved in calculating this fraction via f_P and b_H (see below).

4.3 CHARACTERISATION OF STOICHIOMETRIC AND KINETIC PARAMETERS

Similar to the overview of wastewater characterisation the overview on characterisation of stoichiometric and kinetic parameters will be clarified according to the applied methodology. The focus will, however, only be on different biological methods since physical-chemical characterisation is not very relevant when it comes to characterisation of reactions. As highlighted in the previous section the majority of the processes involves oxygen consumption which means that respirometry will again be the dominating method in the review. However, also other methods such as nitrate utilisation rates, titrimetry and ammonium uptake rate are powerful to assess some of the kinetic and stoichiometric parameters.

4.3.1 Respirometry

4.3.1.1. Stoichiometric parameters By definition, determination of stoichiometric parameters requires the measurement of two factors that are related to the substrate uptake. One of these factors may be the respiration rate. Theoretically, for ASM1 the following stoichiometric parameters can be evaluated using respirometry: Y_H, Y_A, i_{XB} and f_p, though attempts are reported only for the first two.

Heterotrophic yield coefficient Y_H

This parameter not only influences the estimation of sludge production and oxygen demand but also has an impact on the value of other parameters whose determination requires a value for Y_H (see Table 6) An example is the determination of S_S from respirometric data as described above (Eq. 7). Hence, an accurate value for Y_H is of great importance. Y_H can be determined using respirometry by addition of an amount of wastewater COD and measurements of the substrate oxidation $r_{O,ex}$ (Sollfrank and Gujer, 1991; Brands *et al.*, 1994). Eq. 19 is then applied to evaluate Y_H .

$$Y_H = \frac{COD_{degradable} - \int_0^t r_{O,ex}(t)dt}{COD_{degradable}} \qquad (19)$$

The amount of degradable COD ($COD_{degradable}$) is given by the COD concentration in the filtered wastewater minus the inert fraction (S_I). In the study of Sollfrank and Gujer (1991) S_I was determined as the soluble COD concentration in the effluent.

Brands *et al.* (1994) and Liebeskind *et al.* (1996) circumvent the problem of determining S_I by using a completely biodegradable substrate (acetate) instead of wastewater. Hence, $COD_{degradable}$ is known exactly. This approach is, however, doubtful. First, the choice of acetate is rather arbitrary and there is quite some evidence that the yield coefficient for acetate differs from the influent wastewaters (Dircks *et al.*, 1999). Hence, acetate is not really representative for wastewater COD. Moreover, due to the experimental conditions in the batch reactor, it can be expected that part of the acetate is stored in the cell (Majone *et al.*, 1999). In this case the observed oxygen demand only

represents the needs for transport of the substrate and incorporation in storage material of the cell, and not for the complete conversion into new biomass. Conclusively, these procedures for estimation of the heterotrophic yield do not seem without problems.

Autotrophic yield coefficient Y_A

A value of 0.24 g biomass COD per g nitrified nitrogen is generally assumed to be a good theoretical value for Y_A. If required it is possible however to determine the actual Y_A from a respirometric batch experiment in which a known pulse of ammonium ($S_{NH}(0)$) is added to a nitrifying activated sludge sample (Eq. 20).

$$Y_A = \frac{4.57 \cdot S_{NH}(0) - \int_0^t r_{O,ex}(t)dt}{S_{NH}(0)} \qquad (20)$$

In this approach care has to be taken that no significant net growth of heterotrophs take place as they would incorporate part of the added ammonium. In the model based data interpretation applied by Spanjers and Vanrolleghem (1995) correction for incorporation of S_{NH} into biomass is taken into account directly via the model.

Nitrogen content of the biomass i_{XB}

Obviously, the most likely method for evaluation of i_{XB} would consist of a nitrogen analysis of the biomass. However, one can imagine (albeit maybe not very realistically) that nitrogen incorporation into biomass can be assessed using two respirometric experiments with nitrifying sludge in which different amounts of COD are degraded, the difference being denoted as $\Delta COD^{Degraded}$. The reduction in the oxygen consumption for nitrification $\Delta \int r_{O,ex}^{N}(t)dt$ that can be observed for the higher COD loading then allows a calculation of i_{XB} (development of Eq. 9).

$$i_{XB} = \frac{Y_H}{4.57 - Y_A} \cdot \frac{\Delta COD^{Degraded}}{\Delta \int r_{O,ex}^{N}(t)dt} \qquad (21)$$

Inert particulate fraction of the biomass f_P

Decay of biomass results in a fraction being transformed into inert particulate products. Typically 20 % of the biomass consists of inert material (Henze *et al.*, 1987). This inert biological fraction is called f'_P. The model f_P can be calculated starting from the biological f'_P with the following implicit equation:

$$f'_P = \frac{f_P}{1 - Y_H \cdot (1 - f_P)} \qquad (22)$$

If the studied activated sludge has a yield coefficient (estimated for instance by using respirometry) deviating from the one reported in literature, the f_P-value must be adapted for this. Keesman *et al.* (1998) theoretically showed that the value of f_P can be estimated directly from a batch test in which only the evolution of the respiration rate and sludge concentration are monitored over sufficiently long time.

4.3.1.2. Kinetic parameters Basically the kinetic parameters that can be determined via respirometry are related to aerobic growth, decay and nitrification.

Heterotrophic decay coefficient b_H

The classical respirometric method for determination of b_H' described by Henze *et al.* (1987) is the protocol proposed by Marais and Ekama (1976) and is the most typical method applied for the determination of the decay coefficient (e.g. Sollfrank and Gujer, 1991; Kappeler and Gujer, 1992). Sludge is inhibited for nitrification and is aerated in a non-fed batch reactor. The (endogenous) respiration rate is measured at certain time instants over a period of several days. Since the endogenous respiration is proportional with the active biomass concentration, a plot of the logarithm of the endogenous respiration rate $r_{O,end}$ as function of time describes the exponential biomass decrease as a straight line with slope b'_H.

The death regeneration concept implies that the classical methods for determination of the decay of biomass based on endogenous decay can not be applied directly. The parameter based on the endogenous decay concept has to be translated to the death regeneration concept, similarly to f_P (Eq. 22), leading to the ASM1 decay coefficient b_H (Eq. 23).

$$b_H = \frac{b'_H}{1 - Y_H \cdot (1 - f_P)} \tag{23}$$

Hence, the stoichiometric parameters Y_H and f_P are necessary for calculation of b_H.

Vanrolleghem *et al.* (1992) describe a fast method for estimation of b'_H using only one measurement of the endogenous respiration (in absence of nitrification) in a batch reactor. By means of Eq. 24 describing endogenous respiration, b_H can be calculated on condition that f_p and X_{BH} are known.

$$r_{O,end} = (1 - f'_P) \cdot b'_H \cdot X_{BH} \tag{24}$$

The estimation of b'_H can also be based on the fact that the respiration rate for substrate oxidation is proportional to the heterotrophic biomass concentration (Spanjers and Vanrolleghem, 1995). If a sufficiently high amount of oxygen S_O and substrate S_S are present, $r_{O,ex}$ is not substrate limited and will only be proportional to X_{BH}. Consequently, the decay of the heterotrophic biomass can be determined by (i) taking a sludge sample from the aerated and non-fed batch reactor at certain time instants (t_k), (ii) adding a sufficient amount of substrate and (iii) measuring the maximum respiration rate. Assuming that Y_H and μ_{maxH} remain constant during incubation, plotting the logarithm of $r_{O,ex}(t_k)$ as function of time again allows to determine b'_H as the slope of the curve

obtained via linear regression. In the study of Spanjers and Vanrolleghem (1995) a model-based interpretation was applied to obtain accurate values of the maximum respiration rates. However, only two data points were used for the semilog regression, which does not make the estimated decay coefficients in this study very reliable.

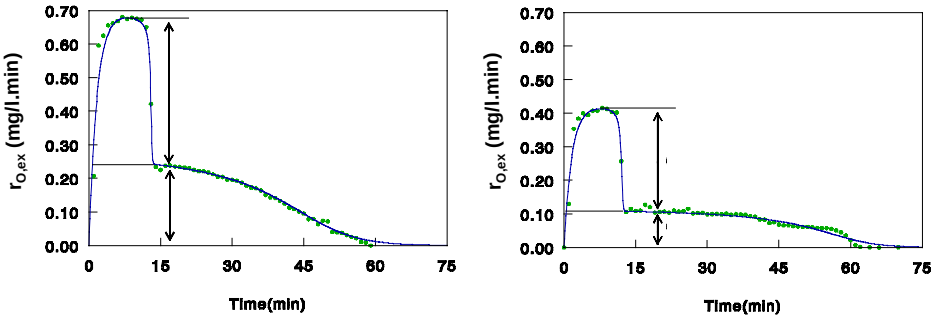

Fig 17. Respirograms obtained after injection of a C/N mixture for the simultaneous determination of b_H and b_A according to the procedure of Spanjers and Vanrolleghem (1995). Left: after 1 day incubation, Right: after 7 days

In the study of Avcioglu *et al.* (1998) a similar procedure was developed, where the decay rate $b'_{H'}$ was assessed by monitoring the decrease in maximum respiration rate. Avcioglu *et al.* (1998) included more data points compared to the study of Spanjers and Vanrolleghem (1995). It was proposed that this method of determining the decay rate should be more reliable, since interference of slowly biodegradable substrate, especially in the initial phase of the traditional test of Marais and Ekama (1976), and inaccuracy of low endogenous respiration rate measurements were avoided. The latter will, however, evidently depend on the sensitivity of the applied respirometric method.

Furthermore, in the work of Avcioglu *et al.*, (1998) it was experimentally verified that the anoxic heterotrophic decay rate was reduced with about 40-50% compared to aerobic conditions. Other studies confirm the observation that the heterotrophic decay is slower under anoxic conditions (McClintock *et al.*, 1988; Siegrist *et al.*, 1999).

Autotrophic decay rate coefficient b_A

The death regeneration concept is not applied for the autotrophic biomass in ASM1. However, the approach of monitoring the decrease in $r_{O,end}$ as function of time can not be applied for the determination of b_A since that would require for instance an inhibition of the heterotrophic biomass. Instead, the method based on the maximum substrate (here S_{NH}) degradation rate as function of time can be applied similar to the procedure for the heterotrophic decay coefficient. In fact, in the procedure described by Spanjers and Vanrolleghem (1995) the heterotrophic and autotrophic decay rate coefficients were determined simultaneously by addition of a mixture of acetate and ammonium. Fig. 17 shows the $r_{O,ex}$ data for the two respirometric tests performed after one and seven days of sludge incubation, clearly illustrating the decreasing activity.

Nowak *et al.* (1994) pointed to the fact that the release of nitrogen due to decay of heterotrophic biomass may result in some growth of nitrifying organisms. Hence, an

underestimation of b_A would result. To correct this, they proposed the incubation of the sludge under anoxic conditions to prevent growth of nitrifiers. Daily a sludge sample was removed from the anoxic reactor and (after aeration) the maximum respiration rate was determined. It was however observed that the reduction in maximum respiration rate was significantly smaller (about 50%) under anoxic than aerobic conditions. This was further confirmed by work on immobilized *Nitrosomonas* (Leenen *et al.*, 1997) and by the findings of Siegrist *et al.* (1999).

Maximum specific heterotrophic growth rate μ_{maxH} and half-saturation concentrationt K_S
The maximum heterotrophic growth rate μ_{maxH} can easily be determined from the maximum $r_{O,ex}$ (Eq. 25) (Ekama *et al.*, 1986), assuming that the substrate concentration is in excess and the yield coefficient and heterotrophic biomass concentration (see previous section) are known.

$$\mu_{max\,H} = \frac{r_{O,ex} \cdot Y_H}{(1 - Y_H) \cdot X_{BH}} \qquad (25)$$

However, the methodology proposed by Ekama *et al.* (1986) does not provide information on K_S.

The increase of the substrate uptake rate with increasing S_S concentration is depicted in Fig. 18. From such Monod type evolution the maximum specific growth rate μ_{maxH} and the half-saturation constant K_S can be determined. In Cech *et al.* (1984) a respirometric method is described in which a number of measurements are performed, each of which add one point to Fig. 18. In this procedure experiments are carried out with addition of different amounts of wastewater (substrate) to endogenous sludge, allowing to achieve various substrate uptake rates, i.e. exogenous respiration rates ($r_{O,ex}$), up to a maximum rate.

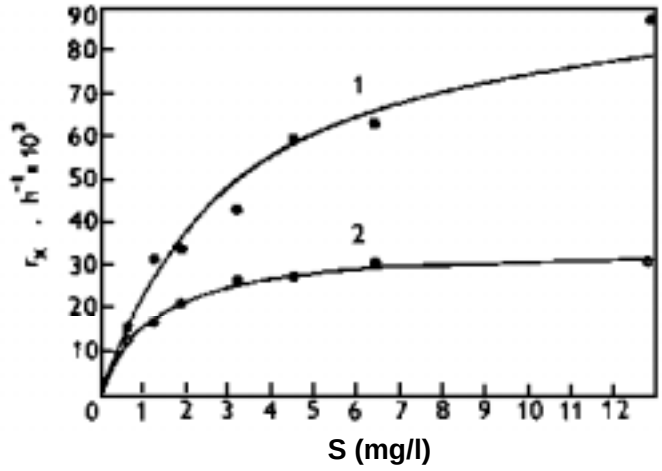

Fig 18. A plot of substrate uptake rate versus substrate concentration for estimation of the parameters for growth, example with valeric acid (Cech et al., 1984)

The parameters μ_{maxH} and K_S can, for instance, be found by Lineweaver-Burk linearisation of Eq. 26 that describes the curve in Fig. 18 (Cech *et al.*, 1984), although the statistical quality of this procedure is not optimal (Robinson, 1985).

$$\frac{dS_S}{dt} = -\frac{\mu_{max\,H} \cdot X_{BH}}{Y_H} \cdot \frac{S_S}{K_S + S_S} \tag{26}$$

The method of Cech *et al.* (1984), which was also applied by e.g. Volskay and Grady (1990), is rather time consuming and the experimental effort is high. As an alternative a more efficient approach was presented, using a continuously aerated respirometer to which a single substrate pulse is added (Vanrolleghem *et al.*, 1990; Kong *et al.*, 1994). In this method $r_{O,ex}$ is recorded frequently as the experiment progresses and one experiment is sufficient for the determination of both μ_{maxH} and K_S provided that the concentration of added substrate is sufficiently high. In this approach a model (Eq. 27 - 28) is fitted to the $r_{O,ex}$ profile for the determination of μ_{maxH} and K_S. An example of an acetate addition is illustrated in Fig. 19 (obtained from Kong *et al.*, 1994) where $r_{O,ex}$ is illustrated together with the corresponding cumulative oxygen consumption and substrate concentrations as function of time.

$$\frac{dS_S}{dt} = -\frac{\mu_{max\,H} \cdot X_{BH}}{Y_H} \cdot \frac{S_S}{K_S + S_S} \tag{27}$$

$$r_{O,ex} = (1 - Y_H) \cdot \frac{\mu_{max\,H} \cdot X_{BH}}{Y_H} \cdot \frac{S_S}{K_S + S_S} \tag{28}$$

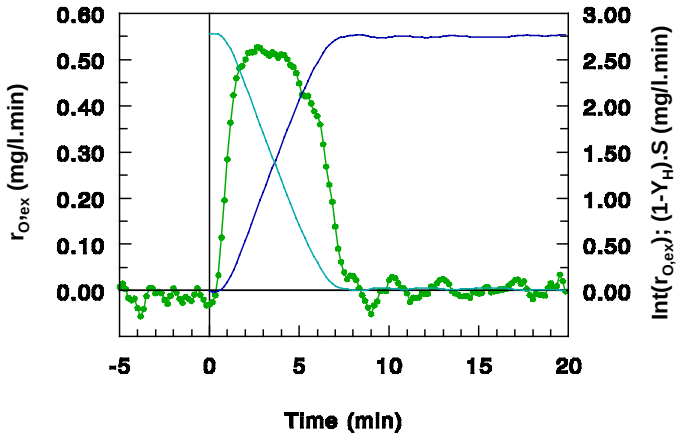

Fig 19. $r_{O,ex}$ (symbols), cumulative oxygen uptake (increasing line) and substrate concentration (decreasing line) in batch experiment (Kong et al., 1994).

The heterotrophic kinetic parameters can also be determined based on the cumulative oxygen uptake profiles rather than oxygen uptake rate data. In the methodology described by Ellis *et al.* (1996) and Smets *et al.* (1996) the kinetics are determined for specific organic chemicals. However, the procedure is directly applicable for wastewaters as well.

A batch experiment with high initial substrate (wastewater) to sludge ratio (called the S(0)/X(0) ratio) was proposed by Kappeler and Gujer (1992). This procedure also enables estimation of μ_{maxH} and K_S from a single experiment. An alternative to the method of Kappeler and Gujer (1992) is to plot the oxygen uptake rate versus the cumulative oxygen consumption (Smets *et al.*, 1996). Fig. 9 shows a respirogram obtained with such an experiment (Kappeler and Gujer, 1992). Contrary to the procedures of e.g. Vanrolleghem and Verstraete (1993) biomass growth is significant and μ_{maxH} can be assessed directly without knowledge of Y_H. A plot of the logarithm of the r_O measurements versus time has the slope (μ_{maxH} - b_H). If b_H is known, a calculation of μ_{maxH} is possible (in the work presented by Kappeler and Gujer (1992), it is assumed that the decay rate is 5% of the growth rate). Attention has to be paid to the fact that the high S(0)/X(0) ratio in this experimental set-up (about 4/1) gives rise to significant growth of the biomass during the experiment. This means that the observed kinetic characteristics may no longer be representative for the original sludge, due to the risk that the experimental conditions may have favoured fast growing organisms that become dominant during the experiment. Novák *et al.* (1994) gave practical evidence for this hypothesis by evaluating results from experiments with different S(0)/X(0) ratios. A 2.5 times higher specific growth rate was obtained at high S(0)/X(0) ratio, compared to an experiment with a low S(0)/X(0) ratio.

In the work of Grady *et al.* (1996) the terminology of intrinsic and extant kinetics was introduced. Intrinsic kinetics refer to the ultimate capacity of the biomass, whereas extant kinetics refer to the biomass activity prior to the lab-scale experiments, e.g in the full-scale plant. This will be discussed further in later sections.

Maximum specific autotrophic growth rate μ_{maxA} and half-saturation concentration K_{NH}

In the studies by Drtil *et al.* (1993) and Nowak *et al.* (1994) the above mentioned methodology of Cech *et al.* (1984) was applied to evaluate the maximum specific autotrophic growth rate and half-saturation concentration K_{NH}. To assess the r_O for autotrophic activity only, the heterotrophic endogenous respiration was determined by a separate experiment, where ATU was added, and was subtracted from the total r_O obtained from an ammonium addition. Here too knowledge of Y_A and X_{BA} is needed for the calculation of μ_{maxA}. In the work by Nowak *et al.* (1994) the concentration of X_{BA} was determined based on full-scale data.

Alternatively, μ_{maxA} and K_{NH} can be obtained directly from experimental data of a simple ammonium addition as presented in Fig. 20. In a study by Spanjers and Vanrolleghem (1995) a model-based interpretation was applied for the determination of the nitrification kinetic parameters (Eq. 29), similar to the approach described above for the kinetic parameters of heterotrophic growth.

$$r_{O,ex} = (4.57 - Y_A) \cdot \frac{\mu_{max\,A} \cdot X_{BA}}{Y_A} \cdot \frac{S_{NH}}{K_{NH} + S_{NH}} \qquad (29)$$

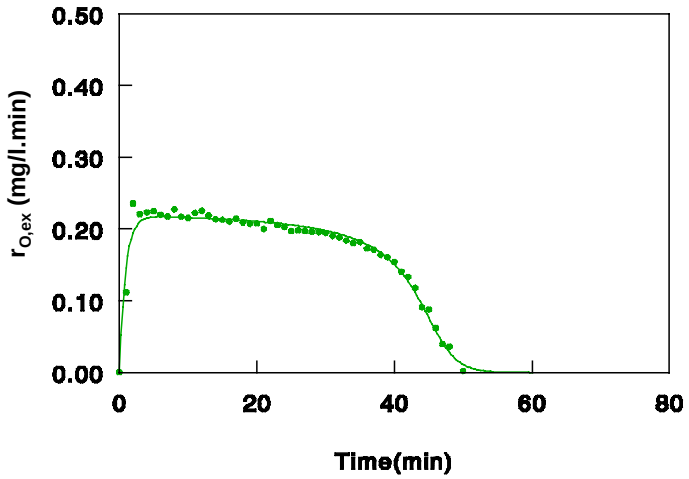

Fig 20. Respirogram obtained after injection of 3.31 mg NH_4-N in 1.4 l activated sludge (Spanjers and Vanrolleghem, 1995)

Hydrolysis constants k_h, K_X

As far as known the only experimental protocol that enables a determination of both parameters of the hydrolysis process is the "cyclic square wave feed" experiment proposed by Ekama *et al.* (1986). This method has already been described earlier for the determination of S_S with a typically obtained profile shown in Fig. 10. To determine the hydrolysis parameters the data obtained after the drop in respiration rate are important. If r_O remains constant on a plateau value (as is noticed in Fig. 10 between t = 12 and t = 15 h), this is related to the hydrolysis that proceeds at maximum rate and the biomass that is saturated with hydrolysable products (X_S /X_{BH} >> K_X). As such, these data contain the information to assess the value of k_h on condition that the heterotrophic biomass concentration X_{BH} and the yield coefficient Y_H are known. With decreasing X_S also the rate of hydrolysis decreases and the respiration rate is depending on the value for K_X, allowing its estimation. Estimation of the parameters is best by means of model optimisation (Henze *et al.*, 1987).

In many cases the dependency of the rate of hydrolysis on the heterotrophic biomass concentration may be neglected and first order hydrolysis process dynamics are then obtained (Sollfrank and Gujer, 1991). This assumes that X_S/X_{BH} << K_X. Sollfrank and Gujer (1991) proposed a method to determine the first order hydrolysis constant, i.e. k_h/K_X, using respiration rates measured by dosage of wastewater to a continuous flow pilot reactor. To simplify the estimation, they suggested to present the respiration rate as function of the residual amount of substrate. In this plot one is able to isolate a linear part from which the hydrolysis constant k_h/K_X is deduced (provided Y_H is known).

For estimation of the first order hydrolysis constant k_h/K_X Kappeler and Gujer (1992) performed a batch experiment with an initial COD based S(0)/X(0) biomass ratio which was 10 times higher than their experiment for determination of the maximum specific growth rate (S(0)/X(0) = 1/2). Fig. 8B shows the respiration rate data of such an experiment, from which the slowly biodegradable substrate, X_S, can also be determined,

as described above. Once the readily biodegradable substrate S_S is removed (in Fig. 8B after 0.75 h) the further decrease of the respiration rate is determined by hydrolysis of X_S. As a consequence, the r_O measurements enable to estimate the hydrolysis rate constant. The authors advise to do this exercise at different biomass concentrations to check for a possible dependency of the hydrolysis rate to the biomass concentration.

Parameters of "switching functions" K_{OH}, K_{OA}

Kappeler and Gujer (1992) determined the respiration rate as function of different oxygen concentrations in the respiration chamber of their respirometer. According to these authors the concentration of readily biodegradable substrate S_S needs to exceed a minimal concentration in order to have an accurate determination of K_{OH}. The same technique can be used for K_{OA} with ammonia as substrate.

Ammonification rate constant k_a

So far, no respirometric method has been reported for the determination of the ammonification rate. However, it is theoretically possible (see Table 6) to assess this parameter from the evolution of the oxygen consumption for nitrification resulting from ammonified nitrogen, provided ammonification is the rate limiting step.

Simultaneous determination of heterotrophic and autotrophic kinetic parameters.

In the previous sections on determination of heterotrophic and autotrophic growth kinetics the focus was put on how to determine the kinetic parameters for the heterotrophic and autotrophic processes separately. However, except for the examples of Sollfrank and Gujer (1991) and Kappeler and Gujer (1992) the presented examples mainly dealt with additions of known substrates (acetate as carbon source and ammonium). The fact is that when dealing with real wastewater and activated sludge both heterotrophic and autotrophic processes will take place simultaneously, and a detailed data interpretation of the respirograms and good experimental design will be needed to "separate" and as such determine the kinetic parameters for the different processes.

Vanrolleghem and Verstraete (1993) proposed an experimental design that enables to simultaneously measure both heterotrophic and autotrophic maximum respiration rates. In their approach a mixture of ammonium and acetate was added to endogenous sludge. The maximum respiration rate for carbon oxidation and nitrification can be derived from the respirograms on the condition that the two aerobic processes can be clearly distinguished from each other. The problem with this approach is however that the kinetic parameters are highly dependent on the nature of the substrate. Thus, the use of a single compound like acetate to represent a complex substrate like wastewater is difficult to justify scientifically.

In the study on wastewater by Spanjers and Vanrolleghem (1995) experiments with municipal wastewater were presented with much lower substrate to biomass ratios (S(0)/X(0)) compared to Kappeler and Gujer (1992). Fig. 21 shows a typical respirogram from an experiment with a S(0)/X(0) of 1/200. This respirogram is much more complicated to interpret than the ones shown so far. First, simultaneous carbon oxidation and nitrification take place. The only seven minutes lasting initial peak in $r_{O,ex}$ is assumed to be due to the oxidation of S_S. After some time only nitrification and,

assumingly, oxidation of substrates released by hydrolysis occurs. In this work the respirograms of the wastewater were interpreted with a more complex ASM1 based model including degradation of two readily biodegradable substrates S_{S1} and S_{S2}, first order hydrolysis and nitrification. Thus, kinetic parameters for all these processes were obtained simultaneously. Experiments in the presence of a nitrification inhibitor ATU were performed to check the contribution of nitrification to the respiration rate. This is shown in the insert of Fig. 21, where the $r_{O,ex}$ related to the degradation of S_S and X_S can be observed.

An approach circumventing ATU addition, suggested by Spanjers and Vanrolleghem (1995), consisted of the following two-step procedure. First, the nitrification process is characterised separately via an experiment where only ammonium was added, as described above and illustrated in Fig. 20. In a second step, the full model is applied to fit to the data of Fig. 21. However, during this step the nitrification parameters are kept at their values obtained from the separate nitrification experiment, and are thereby used to "eliminate" the nitrification oxygen consumption in an experiment with addition of wastewater. The amount of nitrogen in the wastewater sample can be estimated simultaneously as it determines the length of the nitrification shoulder. Spanjers and Vanrolleghem (1995) demonstrated that the ATU and model-based elimination of the nitrification respiration rate lead to similar values for the kinetic parameters and waste water characteristics.

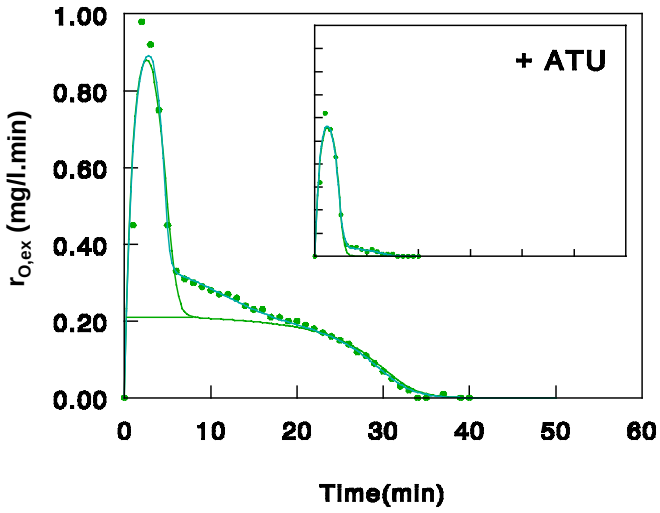

Fig 21. Respiration rate after injection of 70 ml raw waste water to 1.5 l activated sludge. Insertion: similar experiment but after addition of ATU (Spanjers and Vanrolleghem, 1995)

Another example of a detailed interpretation of a respirometric test with municipal wastewater addition is given by Brouwer *et al.* (1998). Here a model including degradation of two readily biodegradable substrates, hydrolysis and two step nitrification is applied to interpret wastewater respirograms. The problem encountered in this study was, however, that not all processes were clearly identifiable from the

respirograms. It was thus suggested that the number of unknown model parameters should be reduced for this example by including experiments with separate additions of synthetic substrates, for example ammonium and nitrite. In this way it would be possible to fix these kinetic parameters in the characterisation of the complex wastewater, similar to the approach of Spanjers and Vanrolleghem (1995).

Finally, an application with industrial wastewater (no nitrification) was presented by Coen *et al.* (1998), where a model-based interpretation approach was applied for the determination of kinetic parameters and substrate concentrations of simultaneous degradation of three COD wastewater fractions.

4.3.2 Nitrate utilisation rates

Characterisation of reaction kinetics via analysis of the nitrate utilisation rate is basically very similar to the methodology based on oxygen respiration rates, and different studies have dealt with the comparison of $r_{O,ex}$ and $r_{NO3,ex}$ (e.g. McClintock *et al.*, 1988; Kristensen *et al.*, 1992; Orhon *et al.*, 1996; Sözen *et al.*, 1998). In ASM1, the same kinetic expressions are applied for nitrate utilisation processes as for oxygen, with the only difference that a correction factor η is incorporated in the equations for anoxic processes. This factor allows to describe that only a fraction of the total biomass is capable of respiring with nitrate and/or that the anoxic rate is lower than the aerobic one. Typically, one applies the relationship given in Eq. 30 in order to relate $r_{O,ex}$ with $r_{NO3,ex}$.

$$\eta = 2.86 \cdot \frac{r_{NO3,ex}}{r_{O,ex}} \qquad (30)$$

Correction factors for anoxic growth and hydrolysis η

It has been shown that the value of η can vary significantly for different activated sludge systems. In different studies values have been recorded in the range 0 - 0.95 (Van Haandel *et al.*, 1981; Henze, 1986; Henze *et al.*, 1987; 1995; McClintock *et al.*, 1988; Kristensen *et al.*, 1992; Sözen *et al.*, 1998; Spérandio *et al.*, 1999). Some theories were developed based on general mass balances that allowed for an estimation of η from wastewater characteristics, treatment plant layout and operation (Henze, 1986). It was shown that the dominating factor for η is the potential inlet fraction of denitrifiers, which includes the denitrifying fraction of the influent biomass plus the primary produced anoxic biomass. Based on some practical constraints concerning e.g. minimum anoxic sludge age and minimum aerobic sludge age to keep both nitrification and denitrification in the system, it was estimated that in practice η might be in the order of 0.4 - 0.9 (Henze, 1986).

An underlying assumption behind Eq. 30 is that the aerobic and anoxic yields are equal. As discussed above significant evidence exists that the anoxic yield may be lower than the aerobic one. In the studies by Orhon *et al.* (1996) and Sözen *et al.* (1998) very high values (>1) for the conversion factor η were related to possible lower anoxic yields for which correction will be needed. The occurrence of lower anoxic biomass yields was already discussed in the section about application of nitrate utilisation rates for the determination of readily or slowly biodegradable substrate S_S and X_S.

4.3.3 Titrimetry

Maximum specific autotrophic growth rate μ_{maxA} and half-saturation concentration K_{NH}

So far the titrimetric technique, based on pH control and monitoring of the cumulative amount of base or acid added to keep the pH set-point, proposed by Ramadori *et al.* (1980), and introduced in more detail above, has only been applied to the determination of the nitrification kinetic parameters μ_{maxA} and K_{NH}. As illustrated in Fig. 12 and 13 and by Eq. 12, the cumulative amount of base added can be used to calculate the nitrification rate and thereby provide kinetic information. In the work of Gernaey *et al.* (1998) a model-based data interpretation was applied for the estimation of μ_{maxA} and K_{NH}. The model is similar to the one applied for the description of respirometric and nitrate utilisation rate data. The only difference is the stoichiometric coefficient relating the ammonium degradation to proton production Hp (Eq. 31).

$$r_{Hp} = \frac{2 + Y_A \cdot i_{XB}}{14} \cdot \frac{\mu_{max\,A} \cdot X_{BA}}{Y_A} \cdot \frac{S_{NH}}{K_{NH} + S_{NH}} \qquad (31)$$

4.3.4 Summary and discussion of biological characterisation of stoichiometric and kinetic parameters

The above review on biological characterisation has illustrated that, theoretically, nearly all parameters can be determined with biological methods. Especially respirometry stands as a powerful characterisation method but other methods too are useful for the characterisation of specific processes, e.g. titrimetry for the characterisation of nitrification and application of nitrate utilisation rates for the determination of the correction factor for denitrification.

One of the challenges in the application of the biological methods is how to interpret and relate the experimental data to the different processes that may take place simultaneously. It is obvious that experiments with addition of known and simple substrate such as ammonium or acetate are easier to interpret in terms of determination of stoichiometric and kinetic parameters than experiments with real wastewater. For example, it is difficult to assess the heterotrophic yield Y_H by experiments with real wastewater, and in some cases it was therefore suggested to determine it from an experiment with known substrate in the form of acetate (Brands *et al.*, 1994; Liebeskind *et al.*, 1996). It has also been suggested to determine the maximum specific growth rate μ_{maxH} based on experiments with acetate in respirometric experiments (e.g. Vanrolleghem and Verstraete, 1993). However, acetate does not represent the actual wastewater very well. As already stressed above it is generally questionable to use a single substrate to represent complex wastewaters. Furthermore, it is a known phenomenon that acetate easily gets directed towards the storage process instead of directly being consumed for growth (Majone *et al.*, 1999). This means that if such data are only interpreted in terms of the growth process, the estimated parameters related to growth will be erroneous. E.g. the stoichiometric growth yield (Y_H) will be overestimated (Dircks *et al.*, 1999). On the other hand, characterisation of the stoichiometric and kinetic parameters for nitrification can be done by respirometric or titrimetric experiments with single additions of pure ammonium.

It is of course advantageous if several parameters (kinetic or stoichiometric) and some wastewater components can be obtained from the same experiment. This was illustrated in studies with municipal wastewater by e.g. Spanjers and Vanrolleghem (1995) and Brouwer *et al.* (1998), and also for an industrial COD removal case (Coen *et al.*, 1998).

In Table 8 (adopted and modified from Vanrolleghem *et al.*, 1999) the experiments described above for characterisation of stoichiometry and kinetics are concisely represented. Attention is drawn to

- The method (respirometry, nitrate utilisation rates, titrimetry)
- The type of reactor set-up (continuous or batch experiment) and the additions performed;
- The requirement for other information collected from other experiments (or assumed);
- Major assumptions made during the interpretation of the data;
- The reference where more information can be found.

From Table 8, it can for example be seen that in the work of Spanjers and Vanrolleghem (1995) with wastewater (reference SV95 and experiment type "B, WW add.") the parameters μ_{maxH}, K_S, μ_{maxA}, K_{NH} and k_h and the substrate components S_S, X_S and S_{NH} could be retrieved from a single experiment.

It will now be attempted to evaluate whether the characterisation approaches of the kinetic and stoichiometric parameters as reviewed for ASM1 can hold for ASM3 too.

As reviewed above, it should be theoretically possible to assess the ammonification rate from respirometric data, provided that ammonification is the rate limiting step. However, in most applications this is not the case making it difficult to quantify the kinetics of ammonification. Furthermore, ammonification does not affect the model predictions significantly, since it is usually a fast process. Thus, with this in mind the ammonification process was not included in ASM3, thereby also eliminating the need to determine its kinetic rate.

Another simplification in ASM3 is the way the decay process is described. Instead of the more complex death regeneration concept it was chosen to describe decay with a more traditional and simple endogenous decay process. This means that the results from a simple long-term aeration test (Marais and Ekama, 1976), where the endogenous respiration rate is monitored over a period of several days, can be applied more directly. In this way a transformation of the data from the endogenous test to the death regeneration concept is no longer needed. Furthermore, the exclusion of the death regeneration concept also resulted in a simplification of the hydrolysis process, since this process is now only involved in hydrolysis of slowly biodegradable substrate (X_S) contained in the influent.

However, with the introduction of the storage model concept it becomes difficult to separate between the kinetics of storage and growth. Already in the discussion of wastewater characterisation it was pointed out that the yield obtained from a respirometric test is composed of two factors $Y_{net}=Y_{STO}\cdot Y_H$. Furthermore, it does not seem clear how to differentiate between the storage rate and growth rate from e.g. a respirometric test.

Table 8. Overview of biological methods for estimation of ASM1 parameters. (Fields with grey background indicate that respirometry is either not applicable or not relevant)

	Method	Type of experiment	Additional information	Assumptions	References
Stoichiometric parameters					
Y_H	R	B, WW add. B, Ac add.	S_I	 Ac representative of S_s	SG91 Br94
Y_A	R	B, NH_4 add. B, NH_4+Ac add.	 i_{XB}, Y_H	$i_{XB}\cong 0$	SV95 SV95
f_P	R	B	MLVSS		K98
i_{XB}	R	B, COD add.	Y_H, Y_A, ΔCOD^{Deg}		this paper
i_{XP}					
Kinetic parameters					
μ_{maxH}	R	B, n S,Ac adds. B, S, Ac add. B, WW add. B, WW add.	Y_H, X_{BH} Y_H, X_{BH} b_H Y_H, X_{BH}	 μ_{maxH} represent original X_{BH}	C84; VG90 K94; E96; Sm96 KG92 SV95, B98
K_S	R	B, n S,Ac adds. B, S, Ac add. B, WW add. B, WW add.	Y_H, X_{BH} Y_H, X_{BH} b_H Y_H, X_{BH}	 K_S represent original X_{BH}	C84; VG90 K94; E96; Sm96 KG92 SV95, B98
K_{OH}	R	B, n S_O	S_O	S_S sufficiently high	KG92
K_{NO}					
b_H	R	B, no add. B, n Ac add. B, no add.	f_P, Y_H f_P, Y_H	 Y_H , μ_{maxH} constant	ME76; SG91 SV95 V92
μ_{maxA}	R	B, n NH_4 adds. B, NH_4 add.	Y_A, X_{BA} Y_A, X_{BA}		C84 D93; SV95
	T	B, NH4 add.	Y_A, X_{BA}		G98
	A	B, NH4 add.	Y_A, X_{BA}		K92
K_{NH}	R	B, n NH_4 adds. B, NH_4 add. B, WW add.	Y_A Y_A Y_A		N94 D93; SV95 SV95, B98
K_{OA}	R	B, n S_O	S_{NH}	S_{NH} sufficiently high	KG92
b_A	R	B, NH_4 add. B, n NH_4+Ac add.			H87; N94 SV95
η_g	R+N	B, WW add.			K92; S99
k_a	R	B, S add.			This paper
k_h	R	C (on/off) C, WW add. B, WW add.	Y_H, X_{BH} Y_H	max. hydrolysis rate K_X very large K_X very large	E86 SG91 KG92; SV95; B98
K_X	R	C (on/off)	Y_H, X_{BH}		E86
η_h	R+N	B, WW add			K92

Method:
R: Respirometry
N: Nitrate respiration test
A: Ammonia uptake test
T: Titrimetry

Type of experiment
Ac: acetate
B: batch reactor
StSt: steady state
add.: addition
adds.: additions
C: continuous system
WW: waste water
S: synthetic substrate

B95: Bjerre *et al.*, 1995
B97 Bogaert *et al.*, 1997
B94 Brands *et al.*, 1994
B98 Brouwer *et al.*, 1998
C84 Cech *et al.*, 1984
D96 Dhaene, 1996
D93 Drtil *et al.*, 1993
DS94 Dupont and Sinkjaer, 1994
E86 Ekama *et al.*, 1986
E96 Ellis *et al.*, 1996
G97 Gernaey *et al.*, 1997
G98 Gernaey *et al.*, 1998
H87 Henze *et al.*, 1987
KG92 Kappeler and Gujer, 1992
K98 Keesman *et al.*, 1998
K94 Kong *et al.*, 1994
K92 Kristensen *et al.*, 1992
KK99 Kujawa and Klapwijk 1999

L92 Lesouef *et al.*, 1992
ME76 Marais and Ekama, 1976
M95 Massone *et al.*, 1995
N98 Naidoo *et al.*, 1998
N94 Nowak *et al.*, 1994
R97 Rozzi *et al.*, 1997
Sm96 Smets *et al.*, 1996
SG91 Sollfrank and Gujer, 1991
SV95 Spanjers and Vanrolleghem, 1995
S99 Spérandio *et al.*, 1999
S96 STOWA, 1996
U97 Ubisi *et al.*, 1997
U98 Urbain *et al.*, 1998
V92 Vanrolleghem *et al.*, 1992
VV93 Vanrolleghem and Verstraete, 1993
VG Volskay and Grady, 1990
We95 Wentzel *et al.*, 1995
Wi96 Witteborg *et al.*, 1996

4.4 IS CHARACTERISATION VIA LAB-SCALE EXPERIMENTS RELEVANT?

In previous sections the sources of information that can be used for calibration of ASM1 were reviewed and attention was especially focused on how to characterise the different wastewater components, stoichiometric and kinetic parameters. Different problems were already highlighted.

The focus is now turned back to calibration of ASM1 and the aim of describing a full-scale WWTP. It should be remembered that the purpose of the model calibration determines the degree of detail of the information that is needed, e.g. which wastewater components and parameters need a more accurate determination than others. Even though it may be possible to characterise some components or parameters, it may not always be relevant for the actual purpose.

However, the problem is not only whether it is possible to carry out a characterisation of the different model components or parameters in lab-scale. A probably more relevant question is whether it is possible to transfer the lab-scale observations to the full-scale system. Or, to apply the terminology suggested by Grady *et al.* (1996), do the lab-scale experiments provide extant kinetic parameters, i.e. parameters representative for the biomass prior to the experiments? Furthermore it will be discussed how the relations are between lab- and full-scale observations, and how the biological processes are presented in ASM1:

- Transferability between lab-scale and full-scale observations: Are the different components and parameters that may be determined via lab-scale experiments representative, i.e. transferable to the full-scale system? That is, do the experiments provide extant kinetic parameters?
- Transferability between full-scale observations and modeled processes: Are the full-scale processes described in a biologically realistic way in the model or are the model processes lumping different biological processes? If so, it may be impossible to characterise them by any experiment.
- Transferability between model processes and lab-scale observations: Are the processes defined in the model reflected by the lab-scale experiments?

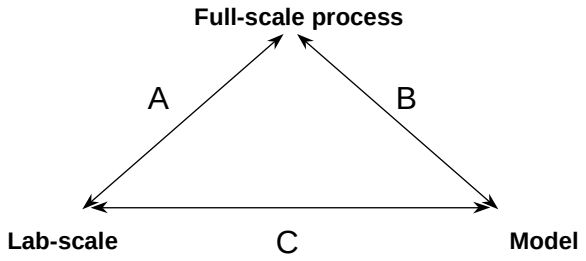

Fig 22. Schematic representation of discussion on transferability

These conflicts of transferability are illustrated in Fig. 22, and the discussion is taken below considering the different wastewater components, kinetic and stoichiometric

parameters. The aim of this discussion is to decide which information source is most relevant for the different components and parameters. Of course, in principle all components and parameters can be obtained from the model, e.g. via the default parameter set or via adjustment of the values during the model calibration exercise.

However, some model processes do not reflect reality completely, although they enable a mathematical description of the biological observations. The model components and parameters related to such processes can not be characterised reliably via either lab-scale or full-scale data and should preferably be tuned during the model calibration with the full ASM1. Then there are some components and parameters that readily and reliably can be transferred from a lab-scale experiment. For others the lab-scale results are difficult to transfer to the model of the full-scale system, and for instance a mass balance with full-scale data may be more appropriate as information source. Whether a certain component or parameter should be obtained via lab-scale or full-scale data or should be tuned directly via the model will depend on what the component or parameter in question is depending on. In this discussion it is assumed that the values of the components and parameters can depend on either the actual biomass in the activated sludge system or the actual WWTP operation. It should be stressed that only the actual state of the system is considered in this discussion, since this is what the calibrated model is aimed at describing. Obviously, the biomass character (e.g. maximum specific growth rate, decay rate etc.) of the WWTP is determined by both the incoming wastewater and WWTP operation. However, changes in biomass characteristics caused by changing WWTP operation or wastewater character are more long-term effects. Description of these effects is not within the scope of the ASM models. Thus, the actual wastewater considered for the model calibration is assumed to be representative for the general wastewater composition to which the biomass has adapted and by which the biomass character is determined.

4.4.1 Kinetic and stoichiometric parameters

Below, the information sources for the most relevant kinetic and stoichiometric parameters will be discussed in relation to Fig. 22. Furthermore, the discussion on whether a parameter is depending on the wastewater, biomass and/or WWTP operation is summarised in Table 9. Finally, the most relevant information source is indicated in Table 9. Brackets in Table 9, i.e. (X), indicate that a lab-scale experiment is possible for determination but the transferability of the obtained parameters to the full-scale situation is uncertain for different reasons, as explained below. Finally, as mentioned above all parameters can in principle be determined based on the model alone without additional supporting information.

<u>The maximum specific heterotrophic growth rate, μ_{maxH}</u>

The observed actual specific growth rate in the full-scale system, μ'_{maxH}, depends on the sludge age and therefore depends both on the actual wastewater and the WWTP operation. If the wastewater contains a significant amount of biomass, μ'_{maxH} will depend primarily on the wastewater, whereas it will depend on the operation if the biomass is primarily produced within the plant. On the contrary, the maximum specific growth rate, μ_{maxH}, is the maximum possible specific growth rate of the actual sludge, and is only influenced by the actual kind of bacteria present. It may be important to

distinguish here between μ_{maxH} and the growth rate, μ, which is influenced by the mixed liquor substrate concentration. Thus, μ_{maxH} is not depending on the wastewater whereas μ is.

Table 9. Discussion on relevant information sources for kinetic and stoichiometric parameters. A bracketed X indicates that a lab-scale experiment is possible for determination but the transferability of the obtained parameters to the full-scale situation is uncertain (see text for further explanation)

	Dependency		Relevant information source		
	Sludge/biomass	Plant operation	Lab-scale experiment	Full-scale data Mass balances	Model calibration
$\mu_{max\,H}$	X		X		X
μ_{maxA}	X		X		X
K_S, K_{NO}	X	X	(X)	(X)	X
K_{NH}	X	X	(X)	(X)	X
K_{OH}, K_{OA}	X	X	(X)	(X)	X
b_H,b_A	X		X		X
Y_{maxH}	X		(X)		X
Y_{maxA}	X		(X)		X
k_h	X				X
K_X	X				X
η_g	X		X	X	X
η_h	X				X

This means that the problem of transferability between the lab-scale and the full-scale observations will be insignificant (conflict a in Fig. 22) if the lab-scale experiment is carried out under conditions that are comparable to the full-scale system (e.g. with respect to pH, temperature, ratio between substrate and biomass concentration etc.). In other words, if the lab-scale experiments are performed in a way that allows measurement of extant parameter values, then little or no conflict will arise. As described earlier, the death regeneration concept in the model has the effect that the cell mass turnover rate increases, resulting in a higher growth rate than if a more traditional concept of endogenous decay was applied. Thus, this should be taken into account in the interpretation of lab-scale experiments and in the transferability of results to the full-scale model (conflict C in Fig. 22). Similarly the death regeneration model concept and the way it influences the maximum specific growth rate may not reflect the full-scale process completely (conflict B in Fig. 22), but may allow for an adequate description of observations.

Summarising, the μ_{maxH} is one of the most relevant parameters to study in lab-scale experiments and can be considered to be a biological parameter, which is only determined by the actual bacteria present (see Table 9).

The maximum specific autotrophic growth rate, μ_{maxA}

Although specific bacterial groups undertake nitrification, they adapt to the actual environment and the bacterial species can therefore vary. Therefore, the discussion on the maximum specific autotrophic growth rate μ_{maxA} is rather similar to the one of μ_{maxH}.

Thus, it is possible to determine the value of μ_{maxA} from lab-scale experiments, and transfer the value to the model of the full-scale system.

Half-saturation coefficients: K_S, K_{NH}, K_{NO}, K_{OA} and K_{OH}

In pure cultures the half-saturation coefficients can be regarded as pure biological parameters that give measures of the affinity of the biomass for substrates. However, in cultures where the bacteria grow in flocs (as in activated sludge), the floc size and structure play a role in the diffusion of substrate to the cell and thereby on the apparent value of saturation coefficients. Especially in full-scale systems mixing characteristics will further influence the apparent values. Even in lab-scale tests under simpler mixing characteristics, mixing may play a role and influence the obtained values of the half-saturation coefficients. Thus, the different mixing characteristics of the lab-scale and full-scale system make it difficult to transfer the lab-scale observation to the full-scale system (conflict A in Fig. 22). If the floc size decreases due to e.g. a more intensive mixing in the small batch-scale experiments, the obtained coefficients will be smaller than required to describe the full-scale behaviour (Henze *et al.*, 1999). This makes it difficult to obtain a model relevant value of the half-saturation coefficients from lab-scale experiments (conflict C in Fig. 22). The saturation coefficients in ASM1 describing a full-scale situation may therefore be regarded more as model parameters with the purpose of preventing unrealistically high substrate uptake and growth rates. Thus, the biological meaning of the model half-saturation coefficients is mixed with the hydraulics of the system (conflict B in Fig. 22). Obviously, if a very detailed model is available to describe the hydraulics of a system accurately, it may be possible to separate the effects of biomass affinity for a substrate and the hydraulic effects from mixing. However, usually the hydraulic pattern is approximated by a simple tanks-in-series model that may be sufficient for a mathematical description but not accurate enough for a complete elimination of hydraulic effects on the biological parameters.

Thus, all half-saturation coefficients of the full-scale system will depend on both the WWTP operation (mixing) and the actual kind of biomass present. The coefficients can be determined by lab-scale experiments but the values obtained may not be very representative. It may therefore be better to estimate these parameters from full-scale data, via the operational rate of COD removal found by mass balances as function of the operational range of COD concentrations. The question is of course whether the full-scale data is informative enough for such determinations. Thus, in practice these values may have to be tuned during the model calibration.

Decay rate of heterotrophs b_H and autotrophs b_A

The decay rate in a full-scale WWTP is in principle a characteristic of the actual biomass, and can, similarly to the maximum specific growth, rate be considered as a biological parameter. However, it may be difficult to obtain a representative value of the decay rates of a full-scale system from the lab-scale tests presented above (conflict A in Fig. 22), since decay and growth due to substrate inflow (and internal production) take place simultaneously in the full-scale WWTP. On the contrary, decay is typically investigated under starving conditions (endogenous respiration) in lab-scale experiments. Furthermore, the decay rate in the full-scale plant is typically influenced

by grazing, i.e. presence of protozoa, which may not be present or may not be able to survive in the lab-scale experiment.

In ASM1, the death regeneration concept includes both lysis combined with hydrolysis of released substrate and, subsequently, growth on this substrate. As discussed earlier, this interaction of different processes makes it difficult to determine the decay coefficient related to the death regeneration concept (conflict B in Fig. 22). However, according to ASM1 it is possible to transfer the decay rate obtained from a lab-scale experiment with decreasing endogenous respiration as function of time for determination of the endogenous decay rate to the death regeneration model concept (via Eq. 23, conflict C in Fig. 22). Obviously, the change in ASM3 to the endogenous respiration decay concept makes it more straightforward to determine the decay rate of the model by a lab-experiment.

In conclusion, it is possible to determine the decay rate via lab-scale experiments, and to convert the obtained value to the death regeneration concept of ASM1, but the value may to some extent have to be adjusted during the model calibration procedure.

Maximum heterotrophic and autotrophic yield, Y_H and Y_A

The observed yields in a full-scale WWTP, Y'_H and Y'_A, are depending on the process operation, i.e. the actual wastewater load and the sludge age. On the other hand, the actual maximum yields (Y_H and Y_A) are depending on the kind of biomass present. For municipal WWTP's the parameters Y_H and Y_A are typically assumed to be rather constant, indicating that the biomass character is rather similar among different municipal WWTP's. However, it may still be needed in some cases to determine the biomass yields. This can be carried out in lab-scale experiments, but there may be some experimental difficulties, e.g. caused by the possible influence of storage which may be induced by the conditions in the lab-scale experiment (Majone *et al.*, 1999), as earlier described (conflict A in Fig. 22).

In fact the typical maximum heterotrophic yield of 0.67 for municipal wastewater (Henze *et al.*, 1987) is higher than the yields observed with pure cultures (Heijnen *et al.*, 1992). The reason for this may be that the model yield covers different processes as storage, death regeneration etc. and may thereby be considered more as a model yield (van Loosdrecht and Henze, 1999) (conflict B and C in Fig. 22). Although the heterotrophic yield seems influenced by the available electron acceptors (the anoxic yield is reported to be lower than the aerobic one, Koike and Hattori, 1975; Orhon *et al.*, 1996; McClintock *et al.*, 1998; Spérandio *et al.*, 1999), the yield may be more influenced by storage than by the electron acceptor.

Hydrolysis rate k_h and half-saturation coefficient K_X

Although only limited knowledge is available about hydrolysis, the process is needed in ASM1 to describe the degradation of slowly biodegradable organic matter originating from the influent COD and from internal turnover of substrate in the death regeneration cycle.

As described above attempts have been made to analyse hydrolysis in lab-scale experiments. It may be possible to compare the real enzymatic hydrolysis as it takes place in lab-scale with the full-scale hydrolysis process. However, the real enzymatic hydrolysis is not the same as the hydrolysis process in the model, as it might also cover

consumption of storage polymers, hydrolysis of decayed biomass (death regeneration), protozoan activity etc. (conflict B and C in Fig. 22) (van Loosdrecht and Henze, 1999). Thus, it remains problematic to design an experiment that is representative for both the model concept and the hydrolysis process as it takes place in full-scale. If this is compared to the determination of e.g. the maximum specific growth rate, we note that this parameter also covers many details but still only describes one process, i.e. growth.

In conclusion, the real hydrolysis process is probably determined by the actual biomass which produces the enzymes, but for the model calibration of ASM1 it does not seem relevant to attempt to characterise this process via lab-scale tests. Hence, the hydrolysis as it is described in ASM1 should be considered as a model process that has to be adjusted during the model calibration procedure. It should be remembered that the definition of hydrolysis has changed in ASM3 and is closer to the real biological hydrolysis. Thus, a characterisation of the hydrolysis parameters from a lab-scale experiment will be more relevant for ASM3. The problem remains, however, to design a good experiment for characterising the real biological hydrolysis.

Correction factors for denitrification η_g and η_h

The correction factors for denitrification can be found via a combination of respirometric and nitrate utilisation rate experiments for the determination of the growth and hydrolysis process, although some problems may be encountered in the case where the aerobic and anoxic yields can not be considered equal. It was also referred above that the correction factors can be determined based on some general mass balances of the full-scale system (Henze, 1986). Both correction factors will depend on the actual biomass character. However, no particular conflicts, as indicated in Fig. 22, are apparent concerning the correction factor for growth, η_g. Determination of the correction factor for hydrolysis will suffer from the same problems as indicated above for the hydrolysis itself, and may therefore also be considered more as a model parameter.

4.4.2 Relevant kinetic and stoichiometric parameters for lab-scale characterisation

In the discussion on the relevance of characterising the stoichiometric and kinetic parameters of ASM1 via lab-scale experiments, one has to remember that none of the ASM model processes are pure or microbiologically correct. To some extent they are all bulk processes. It has clearly been illustrated above that experiments oriented in identifying mechanisms introduced in the model might easily lead to conflict with the actual model coefficients (van Loosdrecht and Henze, 1999). Thus, although possible, it may not always be relevant to retrieve the model parameters from lab-scale tests.

Above the discussion was taken on these conflicts between lab- and full-scale observations and the links to the model processes. Table 9 summarised the dependency of the parameters on the biomass and WWTP operation, and it was attempted to indicate the most relevant information source based on these discussions. Notice the difference to Table 8 that listed how the different parameters could be estimated from lab-scale tests, whereas Table 9 indicates whether this is relevant or not, considering that the parameter should correspond reasonably well both with the full-scale behaviour i.e extant parameters are sought, and with the model concepts.

From Table 9 it is deduced that it may be relevant to determine the following list of stoichiometric and kinetic parameters from lab-scale experiments. It is not judged

whether it is always needed to characterise these parameters since that will depend on the purpose of the model calibration. For the same reason it is not attempted to make an indicative order of parameter importance.

- μ_{maxH}
- μ_{maxA}
- η_g
- b_A
- b_H
- (Y_H)
- (Y_A)

The yields are included in the list, knowing that they are not easy to determine in lab-scale tests and that they are usually assumed to be rather constant. However, it should also be realised that the yield coefficients have an important influence on nearly all the processes (see Table 6), and therefore it would be rather relevant to have a more accurate determination of these.

The remaining parameters can be determined via either full-scale data or directly via the model calibration, as indicated in Table 9. It is important to notice that the above parameter list is significantly reduced compared to the list of parameters retrieved from experiments based on Table 8, basically due to the fact that the half-saturation coefficients and hydrolysis parameters are left out.

4.4.3 Relevant wastewater components for lab-scale characterisation

Only the side of the triangle dealing with the conflict between lab-scale observations and model concepts (conflict C) outlined in Fig. 22 is relevant when it comes to characterisation of wastewater components. Also, the discussion summarised in Table 9 is not relevant here, since the wastewater components do not depend on the biomass or the WWTP operation. Therefore, the discussion on wastewater components is less extensive here (see also the earlier discussion and summary of wastewater characterisation methods) and is not divided according to the different components.

As discussed above in the review of wastewater characterisation a conflict may indeed exist between the need for quantification of some of the ASM1 wastewater components and what is practically obtainable from lab-scale experiments. The origin of this problem mainly lays in the way the components are defined in ASM1. The death regeneration cycle and the hydrolysis processes of ASM1 are model processes that are not directly measurable in lab-scale experiments, as discussed above. Thus, the slowly biodegradable substrate and inert particulate matter components, X_S and X_I respectively, that are related to these processes, may then also be regarded as model components that should rather be quantified during the model calibration exercise than through dedicated experiments. Indeed, it was proposed by Henze *et al.* (1995) to estimate X_I in the influent via the complete model during the calibration of the sludge balance and, subsequently, estimate X_S from the difference between total COD and the other COD components, as discussed earlier. A determination of the heterotrophic biomass (X_{BH}) in the wastewater is possible via lab-scale experiments, as described above. However, in most cases the X_{BH} present in wastewater is not of great importance, since the growth

rates are so high that wash-out of X_{BH} never occurs in practice. Thus, an inclusion of X_{BH} in the X_S component does not affect the modelling significantly, although it will affect the value of the heterotrophic yield coefficient (a slightly smaller yield may need to be chosen) (Henze *et al.*, 1999). On the contrary, the presence of autotrophic biomass (X_{BA}) in the wastewater may be of importance to prevent wash out of the nitrifiers. The concentration of X_{BA} can in principle be determined via lab-scale experiments, but in practice the procedure may not be straightforward and X_{BA} may rather be adjusted during the model calibration.

In general there is no need for a detailed characterisation of the nitrogen components since the main part of nitrogen in wastewater is ammonium without any coupling to the organic matter (Henze *et al.*, 1999). An exception to this may, however, exist for some industrial wastewaters. Thus, the wastewater components relevant to be characterised separately via lab-scale experiments are listed below. Again, an indicative order of importance is not aimed for, since this will depend on the actual case.

- S_{NH}
- S_S
- S_I
- (S_{ND}, X_{ND})

The relevance of determining the inert soluble matter (S_I) is linked to the determination of the soluble readily biodegradable substrate (S_S) since S_I may be needed for the mass balance of soluble COD.

5. Biological experimental constraints

In the previous section the wastewater components and the stoichiometric and kinetic parameters that are considered most relevant to be determined in lab-scale experiments were listed. This list was compiled on the basis of considerations that the component or parameter resulting from the lab-scale experiment should be relevant to full-scale behaviour and fit within the model concepts.

In this last section, we will further zoom in on the problem of transferability between lab-scale results and full-scale behaviour, i.e. the problem of obtaining extant kinetic parameters. As discussed above care should be taken in the transfer of results derived from lab-scale experiments to a model of the full-scale system. Summarising, the reason for problems with transferability are on the one hand differences in biological experimental conditions between lab-scale and full-scale experiments (conflict A in Fig. 22) and, on the other hand, differences in the models used (conflict C in Fig. 22).

At the experimental level the lab-scale behaviour may not equal the full-scale behaviour due to, for instance, differences in feeding pattern resulting in other concentration profiles, differences in environmental conditions such as pH, temperature or mixing behaviour, or differences in sludge history. One of the most discussed biological experimental factors is the ratio between initial substrate concentration (S_O) and initial biomass concentration (X_O). This S(0)/X(0) ratio is considered to be one of the important factors determining (1) the response of the sludge with a certain

wastewater or substrate and (2) whether the experimental response is sufficiently informative for adequate interpretation. The first point is of a more basic nature since it has been observed that the S(0)/X(0) ratio directly influences the behaviour of the sludge, leading to different characteristics (Chudoba *et al.*, 1992; Grady *et al.*, 1996, Pollard *et al.*, 1998). The second point is more related to the practical identifiability of model parameters, i.e. it affects the quality of the experimental data (Spanjers and Vanrolleghem, 1995; Spérandio and Paul, 2000). For instance, if S(0)/X(0) is very high the measured response, e.g. respiration rate, may be too small and the experiment may take too long. On the other hand, if S(0)/X(0) is very low the respirometric response may be too short for a reliable measurement, or it may be swamped into the endogenous respiration rate. Below, special attention is paid to the first point, where the S(0)/X(0) problem will be discussed in more detail.

At the modelling level the results from lab-scale experiments may be described with a model different from the model used to describe the full-scale behaviour. Although not obvious at first sight, the use of a simple model for interpretation of the lab-scale data increases calculation speeds significantly, resulting in, for instance, a faster and more straightforward parameter estimation. Problems arise when the model uses different concepts that may not allow to transfer the estimated parameters from one model to the other, e.g. the death regeneration versus endogenous respiration concept (Yuan and Stenström, 1996).

5.1 TRANSFERABILITY BETWEEN MODEL CONCEPTS: AN EXAMPLE

In ASM1 the death regeneration concept is applied, whereas the model to describe the lab-scale results may only include the degradation of substrates, i.e. decay and death regeneration are omitted, because they are considered insignificant in relation to the time scale used in the experiment (Spanjers and Vanrolleghem, 1995). In ASM1 oxygen is consumed for growth on incoming substrate plus growth on substrate produced due to death regeneration, whereas in a lab-scale model one may only consider that oxygen is consumed for growth on incoming substrate. This is illustrated in Fig. 23.

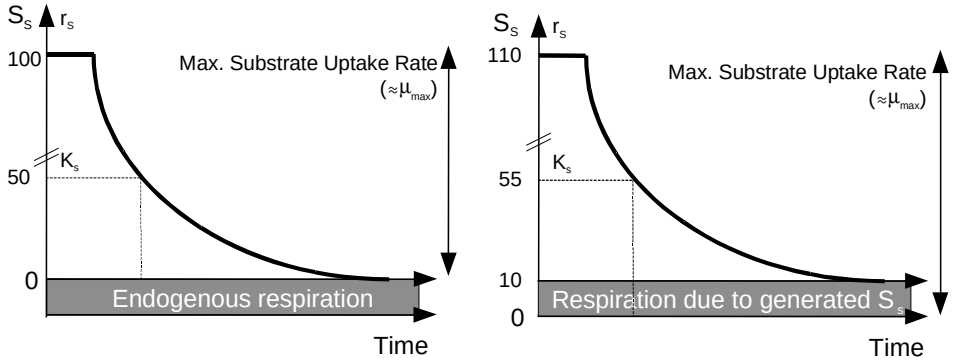

Fig 23. Illustration of difference in interpretation of substrate uptake rate in lab-scale (endogenous respiration, left) model versus ASM1 (death regeneration, right).

In Fig. 23 the line illustrates substrate uptake rate, r_S, as function of time and the values at the left hand side of the y-axes indicate the corresponding substrate concentrations S_S. The left figure illustrates how the substrate uptake rate is interpreted in the lab-scale (batch) experiments whereas the right figure gives the ASM1 interpretation. In both cases the total oxygen consumption rate is the same, but it is interpreted differently in the lab and full-scale model. In the lab-scale model oxygen is consumed to degrade the incoming substrate and the substrate concentration will eventually go to zero. Apart from oxygen for substrate degradation ($r_{O,ex}$), oxygen is also used for endogenous respiration ($r_{O,end}$). In ASM1 substrate will also be degraded. However the concentration will not reach zero since there will be some production of substrate from the death regeneration process. Thus, according to the ASM1 model concept oxygen will be consumed for degradation of both the incoming substrate and the produced substrate. In Fig. 23 it is assumed for clarity that the concentration of the produced substrate is 10 mg COD/l. This slightly higher substrate availability in ASM1 means that the contribution of the observed total r_O to degradation of incoming substrate is lower in ASM1 than in the lab-scale model. As a consequence the estimated maximum growth rate, which is proportional to the maximum r_O, will be lower in the batch system. This is illustrated with the size of the double arrow at the right hand side of both graphs in Fig. 23. Also, the value of the half-saturation coefficient K_S will be underestimated in the batch model compared to ASM1. In the batch model this is illustrated by a K_S value of 50 whereas it may be 55 in ASM1.

As discussed earlier, it is possible to derive analytical transformations between both model concepts for the decay and growth rates, the yield and the fraction of inerts produced (Henze *et al.*, 1987). However, a transformation for K_S is more complicated.

5.2 REVIEW AND DISCUSSION OF S(0)/X(0) RATIO

Depending on the experimental conditions the organic substrate (COD) uptake rate in both lab- and full-scale may consist of different responses. This is illustrated in Fig. 24. In this concept COD is produced from decay (flow 1). Maintenance (flow 2) is defined as the external substrate requirements to maintain the organisms in their current state. Note the difference here to endogenous respiration, which can be defined as the respiration in absence of external substrate (for a detailed review see van Loosdrecht and Henze, 1999). However, here it is assumed that external substrate is present. Growth (flow 3) is divided in two; (i) increase in biomass due to production of cell constituents (e.g. proteins etc.) but without cell multiplication, (ii) increase in biomass caused by cell multiplication. Storage (flow 4) is defined as the accumulation of polymers, e.g. poly-hydroxy-alkanoates and glycogen. Energy spilling (flow 5) (Zeng *et al.*, 1995) is defined as substrate waste that may take place when the organisms are exposed to very high substrate concentrations. In such cases the organisms may not be able to regulate the catabolism rate to the needs for anabolism, resulting in inefficient use of substrate and possible excretion of metabolites. Fig. 24 illustrates the possible COD flows in the single organisms. Depending on the experimental conditions one of the flows may dominate in a single organism (Fig. 24). The same experimental conditions also provoke a particular distribution of COD over the different organisms. Competition may eventually lead to a shift in the population (Novák *et al.*, 1994).

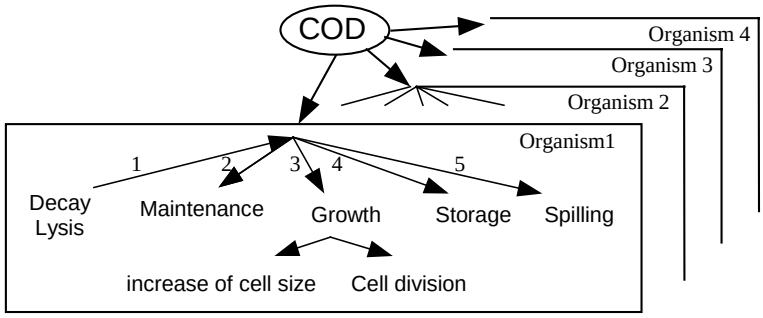

Fig 24. Different flows of external COD in the organisms

As mentioned above the S(0)/X(0) ratio is considered to be one of the determining factors for the way the organisms respond in a system. However, even though the importance of this ratio has been recognised, only few references that deal with the subject in more detail can be found (Chudoba *et al.*, 1992; Novák *et al.* 1994; Zeng *et al.*, 1995; Grady *et al.*, 1996; Liu, 1996). They all deal with the subject from a more theoretical point of view without much experimental support, and there is still a lack of both qualitative and especially quantitative explanation of the exact role of the S(0)/X(0) ratio. The discussion on the effect of S(0)/X(0) can be considered from a reaction stoichiometry or reaction kinetics point of view.

5.2.1 Effect of S(0)/X(0) on stoichiometry

Both Chudoba *et al.* (1992) and Liu (1996) explain the importance of the S(0)/X(0) ratio from a thermodynamic point of view based on the observations that the observed yield (Y'_H) decreased with increasing S(0)/X(0) ratio (Fig. 25).

In the work of Chudoba *et al.* (1992) substrate (COD) profiles versus time were measured. Here it was assumed that autocatalytic growth would cause substrate uptake at an increasing rate whereas substrate uptake at a constant rate was assumed to be an indirect evidence of storage. It was hypothesised that at low S(0)/X(0) ratio the main response is storage (flow 4 in Fig. 24) since the energy level in the cell will be too low to trigger cell multiplication, resulting in less substrate being oxidised (Daigger and Grady, 1982) and thereby a higher Y'_H.

At high S(0)/X(0) ratios on the contrary, the growth response where cell multiplication (flow 3 in Fig. 24) is dominating results in lower observed yields (Chudoba *et al.*, 1992). However, the lower Y'_H at higher S(0)/X(0) ratios may as well be explained by less energy being required for growth without associated cell multiplication (flow 3) and without the involvement of storage (flow 4). A second possible explanation of the data of Chudoba *et al.* (1992) is that the contribution of endogenous respiration to the total amount of oxygen consumed is higher at high S(0)/X(0) ratio. This could also result in lower Y'_H, since the experiments at high S(0)/X(0) ratios take longer time and therefore the amount of decayed biomass (flow 1) is higher.

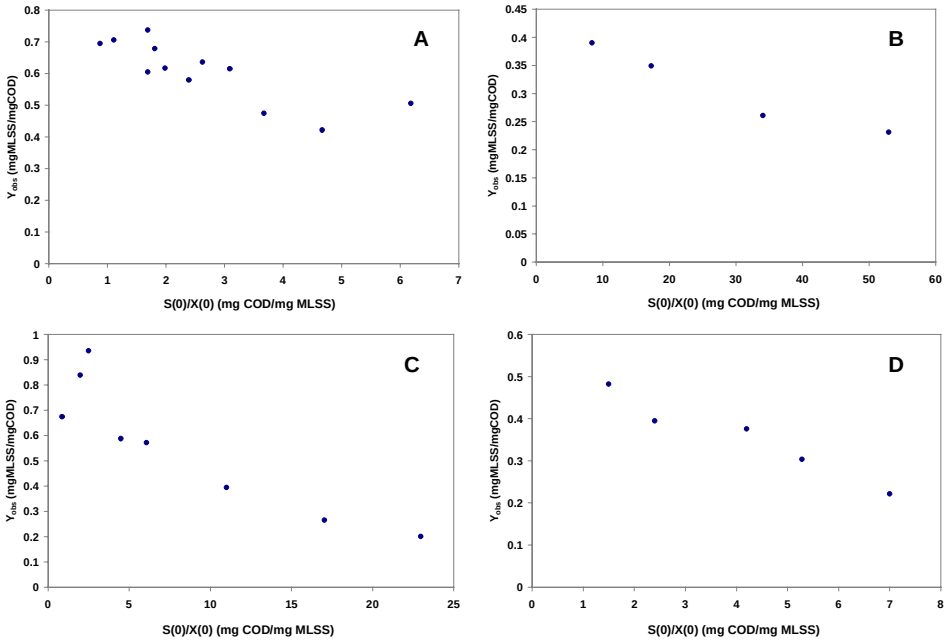

Fig 25. Literature data of Y_{obs} as function of S(0)/X(0) ratio: A: Rao and Gaudy, 1966; B: Chudoba et al., 1969; C: Chang et al., 1993; D: Chudoba et al., 1991. Data digitised from Liu (1996).

A still different explanation of the decreasing observed yield with increasing S(0)/X(0) is found in the work of Liu (1996), who presented an attempt to quantify the importance of S(0)/X(0). Here the decrease in Y'_H is explained by an increase in energy spilling (flow 5) with increasing S(0)/X(0) (Liu, 1996). However, the problem in verifying this approach is to define at which S(0)/X(0) energy spilling will start to take place. In the study of Liu (1996) the ratio is assumed to be 1. The proposed model was tested on literature data, but the S(0)/X(0) ratios of all the literature data used in the study were higher than 1, making the evidence for the model incomplete. It should be noted that none of these studies attempted to explain the observed behaviour with a more complex model, such as ASM1.

5.2.2 Effect of S(0)/X(0) on kinetics

Another way of looking at the influence of S(0)/X(0) is from a kinetic point of view focusing on the physiological, i.e. enzymatic, state and adaptation. In order to describe these phenomena, the concept of the machinery necessary for protein synthesis (PSS) has been introduced (Grady *et al.*, 1996). This should basically be understood as follows. If the organisms are adapted to grow under substrate limited conditions, its PSS will not be sufficient to quickly increase the growth rate if the substrate limitation is removed. Thus, the PSS and eventually the specific growth rate will gradually increase during time, until the maximum possible value according to the new conditions, i.e.

physical adaptation has taken place. It has been stated that the synthesis of storage polymers requires less physiological adaptation than the growth response (Daigger and Grady, 1982). Thus, this would mean that if a substrate limitation is removed, as described above, a storage response may be triggered as a fast response and as an alternative mechanism when the growth response is too slow.

A simple example of physiological adaptation is illustrated in Fig. 26 where three pulses of acetate were added consecutively to a sludge sample (Vanrolleghem *et al.*, 1998). Each of the three responses is characterised by a fast start-up of about two minutes. These two minutes are assumed to be the time needed by a cell to take up fresh substrate and oxidise it (Vanrolleghem *et al.*, 1998). In the first two responses a more gradual increase of r_O is observed for about 10 minutes, presumably due to an increased conversion capacity (e.g. enzyme activation or synthesis). In the third response (after 40 minutes) this capacity has become constitutive. Starvation of the culture for one night turned the capacity down (the organisms "forgot") and a similar behaviour could be observed when acetate was added again (results not shown).

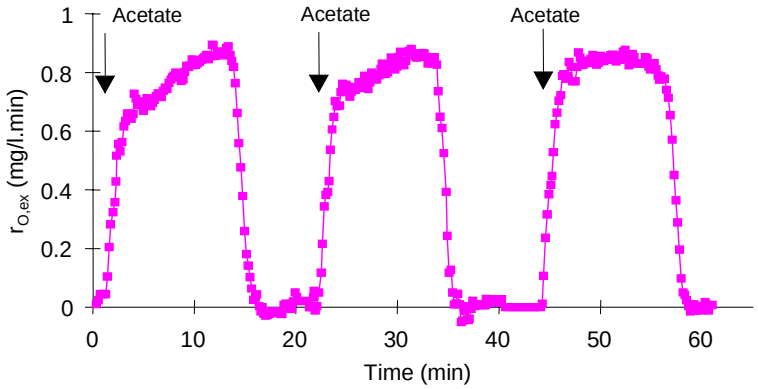

Fig 26. $r_{O,ex}$ profiles obtained by 3 additions of acetate to an activated sludge sample (Vanrolleghem et al., 1998).

In both Chudoba *et al.* (1992) and Liu (1996) the applied S(0)/X(0) ratios are very high (above 1), whereas in the example of Fig. 26 the S(0)/X(0) ratio was very low (below 1/20). It is commonly assumed that it is necessary to work under low S(0)/X(0) ratios (Chudoba *et al.*, 1992; Novák *et al.*, 1994; Spanjers and Vanrolleghem, 1995; Grady *et al.*, 1996). Indeed, if the S(0)/X(0) ratio is high this may result in a change of maximum specific growth and substrate removal rate due to physiological adaptation, which eventually may result in changes of the proportions among slow-growers and fast-growers leading to population shifts (Novák *et al.*, 1994). The kinetics measured under such conditions will more represent the ultimate capabilities of the organisms (intrinsic kinetics), whereas kinetics measured in experiments performed under low S(0)/X(0) ratio may be more representative of the physiological state of the cells prior to the experiments (extant kinetics) (Grady *et al.*, 1996). In the example of Kappeler and Gujer (1992) a very high S(0)/X(0) ratio was applied resulting in overestimation of the growth

rates due to shift in biomass composition towards fast-growers. In addition, population shifts will also take place if the substrate source is changed.

5.2.3 Discussion on S(0)/X(0) ratio

As illustrated above the discussion on the effect of the S(0)/X(0) ratio is looked at from many angles and it seems difficult to draw a coherent picture. However, instead of focusing on a threshold value for the S(0)/X(0) ratio it may be more relevant to consider the following factors in the discussion of what kind of response can be expected in a lab-scale experiment:

- ΔS: how big is the change of substrate concentration in the lab-scale system compared to the full-scale system, i.e. to what extent are organisms subjected to a drastic change in their environmental conditions.
- Time: for how long is ΔS maintained, i.e. what is the time frame of the experiments.
- History: how strong is the history of the sludge, e.g. starvation periods prior to the experiment

These three factors should be understood as follows. If ΔS is low and the experiment is performed over short-term, the risk for changing the response of the sludge compared to the full-scale system is probably low and extant parameters can be obtained. If ΔS is high and the time is short the risk for excess substrate uptake not resulting in immediate growth increases (maybe induction of storage or spilling). Finally, if ΔS is high and the experiment is performed over long-term the risk for physiological adaptation due to enzymatic changes is increasing, eventually leading to a population shift. The specific growth rate may increase during the experiment resulting in an increase in growth response and a decrease in excess substrate uptake response, i.e. the initial stress reaction such as storage or energy spilling will decrease as the organisms get adapted to the new environment. Thus, somehow a compromise between ΔS and time is needed.

Furthermore, the history of the sludge will play a role in the experimental designs, since for example starvation periods prior to the experiment will result in an initial slower response of the sludge. It is, however, not really clear if for example starvation periods can lead to an initial different response.

The above discussion on S(0)/X(0) focused on heterotrophic organisms and their response to a carbon substrate. However, the discussion can easily be extended to autotrophic organisms where the substrate is ammonium. In this case a too high ΔS may result in inhibition of the nitrification process. However, the risk for a population shift may be lower since the nitrifying group of organisms is supposed to be rather uniform in character. Still, adaptations to new environments will take place and the bacterial species can vary.

Summary

In this extensive review numerous aspects of activated sludge model calibration have been touched upon. As an introduction the industry-standard Activated Sludge Model

No. 1 was introduced to set the scene and it was compared to the more recent update ASM3. The wastewater and sludge fractions considered in these models were described and the processes taking place among them were given. All these items are focused upon when calibrating such model.

In a next section an overview was given on the descriptions of calibration procedures that were found in literature. Surprisingly, it is not possible to find a single paper where a comprehensive overview is given. The information is only available as "bits and pieces" and is scattered in a vast amount of literature. The information sets that are typically required were presented and a 10-step calibration procedure was proposed.

The multitude of methods for model calibration was structured along three lines: (1) wastewater characterisation, (2) sludge composition analysis and (3) stoichiometric and kinetic parameters.

The wastewater characterisation is typically done either by physical-chemical or biological characterisation methods. Whereas the former appear the easiest to apply, even in routine lab analysis, their results are not directly related to the model concepts and, moreover, the results need to be augmented with specific characteristics obtained from biological characterisation methods. Among these biological methods attention was particularly given to the respirometric tests as they form the core technique, but nitrate utilisation tests and the upcoming titrimetric tests were presented as well. For the extraction of the model-related information, either direct or model-based analysis is needed. Whereas the former is really simple, the latter allows extracting multiple characteristics from a single experiment.

For the sludge composition analysis, mainly in-out mass balancing methods are being used. The estimation of stoichiometric and kinetic parameters is typically based on dedicated batch experiments using respirometers. Special attention was drawn to the simultaneous estimation of parameters from well-designed single experiments. Especially for this, model-based analysis is required. It is also noteworthy that these more complex approaches not only lead to stoichiometric and kinetic parameter estimates, but typically also lead to estimates on wastewater composition.

In the last section of this review attention was focused upon the problem of transferring the results of the specific tests to a model apt to describe the full-scale behaviour. It was indeed argued that quite some estimation results give a near-perfect description of what happened in the batch test. However this result could not be applied in the practical situation because, for instance, the insufficiently modelled mixing characteristics have to be lumped into the biological parameters of the full-scale model. Still, it was attempted to point towards the parameters whose values can most likely be assessed realistically from lab-scale tests and transferred to the full-scale model.

All in all, this review has led to the belief that a considerable potential exists for efficient characterisation of Activated Sludge Models, provided that precautions are taken with respect to constraining the experimental conditions. The PhD thesis of Petersen (2000) was entirely devoted to this question. The thesis focused on the design of optimal experiments that not only lead to high-information content data sets with good identifiability properties, but that also take into account the biological constraints to guarantee transferability of calibration results to the full-scale model.

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