

Critical review of activated sludge modelling: State of process knowledge, modelling concepts and limitations

Hauduc H.^{a,b}, Rieger L.^c, Oehmen A.^d, van Loosdrecht M.C.M.^e, Comeau Y.^f, Héduit A.^a,
Vanrolleghem P.A.^b and Gillot S.^a

^a Cemagref, UR HBAN, Parc de Tourvoie, BP 44, F-92163 Antony Cedex, France.

^b modelEAU, Département de génie civil et de génie des eaux, Université Laval, Pavillon Adrien-Pouliot, 1065
av. de la Médecine, Quebec (QC) G1V 0A6, Canada.

^c EnviroSim Associates Ltd., McMaster Innovation Park, 175 Longwood Rd S, Suite 114A, Hamilton, ON L8P
0A1, Canada.

^d REQUIMTE/CQFB, Chemistry Department, FCT, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.

^e Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The
Netherlands.

^f Ecole Polytechnique, Department of Civil, Geological and Mining Engineering, P.O. box 6079, Station Centre-
ville, Montreal H3C 3A7QC, Canada.

Abstract

This work critically reviews modelling concepts for standard activated sludge wastewater treatment processes (e.g. hydrolysis, growth and decay of organisms, etc.) for some of the most commonly used models. Based on a short overview on the theoretical biochemistry knowledge this review should help model users to better understand i) the model concepts used; ii) the differences between models and iii) the limits of the models.

The seven analysed models are: (1) ASM1; (2) ASM2d; (3) ASM3; (4) ASM3+BioP; (5) ASM2d+TUD; (6) Barker&Dold model; (7) UCTPHO+. Nine standard processes are distinguished and discussed in the present work: hydrolysis; fermentation; ordinary heterotrophic organisms (OHO) growth; autotrophic nitrifying organisms (ANO) growth; OHO & ANO decay; poly-

hydroxyalkanoates (PHA) storage; polyphosphate (polyP) storage; phosphorus accumulating organisms PAO) growth and PAO decay.

For a structured comparison, a new schematic representation of these processes is proposed. Each process is represented as a reaction with consumed components on the left of the figure and produced components on the right. Standardised icons, based on shapes and colour codes, enable the representation of the stoichiometric modelling concepts and kinetics. This representation allows highlighting the conceptual differences of the models, and the level of simplification between the concepts and the theoretical knowledge. The model selection depending on their theoretical limitations and the main research needs to increase the model quality are finally discussed.

Keywords: ASM, biological nutrient removal, modelling concepts, model selection.

NOMENCLATURE

An: Anaerobic conditions

ANO: Autotrophic nitrifying organisms

ASM: Activated sludge model

ATP: Adenosine triphosphate

Ax: Anoxic conditions

COD: Chemical oxygen demand

BioP: Biological phosphate removal

FADH: Flavin adenine dinucleotide

GAO: Glycogen accumulating organisms

ISS: Inorganic suspended solids

NADH: Nicotinamide adenine dinucleotide

OHO: Ordinary heterotrophic organisms

Ox: Oxic (aerobic) conditions

55 **PAO:** Phosphorus accumulating organisms

56 **PHA:** Poly- β -hydroxyalkanoates

57 **PHB:** Poly- β -hydroxybutyrate

58 **PHV:** poly- β -hydroxyvalerate

59 **PolyP:** polyphosphates

60 **TCA:** Tricarboxylic acid (cycle)

61 **ThOD:** Theoretical oxygen demand

62 **TSS:** Total suspended solids

63 **VFA:** Volatile fatty acids

64 **VSS:** Volatile suspended solids

65 **WWTP:** Wastewater treatment plant

66 **1. INTRODUCTION**

67 Since ASM1 (Henze *et al.*, 1987), a dozen Activated Sludge Models (ASM) and even more
 68 extensions have been published. They have fixed some shortcomings of ASM1 and included
 69 new process insights. Nevertheless, ASM1 remains the most commonly reported model in
 70 literature. Indeed, the results of an international survey among ASM users (Hauduc *et al.*,
 71 2009) revealed that models are found too complex for 22% of the respondents, and that 24%
 72 of the model users do not trust their model. Furthermore, self training is the main source of
 73 knowledge for 78% of model users. Consequently, users are generally not mastering all
 74 published models to be able to choose the most suitable one for their modelling project, and
 75 ASM1 turns out too often be their first choice.

76 Since the first publication of ASM1 (Grady *et al.*, 1986), the biokinetic models are
 77 represented in a table format, which is named in practice Gujer matrix or Petersen matrix
 78 (Takács, 2005). This table contains a stoichiometric matrix and a kinetic vector. Each row of
 79 this matrix stands for a process and each column for a state variable of the model. The

stoichiometric coefficients, negative for consumed compounds and positive for produced ones, are stored in the cells at the intersection of the corresponding row and column. The process rates vector is presented in the rightmost column. This representation is very convenient, as it gathers complex models into a condensed form and facilitates their publication. It also allows seeing at once all state variables involved in a process, and all processes in which a state variable is involved. However, in case of large models such as ASM2d for example, it becomes difficult to "read" this matrix. Comeau and Takács (2008) proposed a schematic representation of ASM, in which each model is represented in a single scheme. This allows a global view of the model processes and their interactions, which is very helpful as a learning tool to understand the models. However, as processes are illustrated in different schematics and as stoichiometric and kinetic information are not represented, it is difficult to compare modelling concepts used in different models, and thus to compare models in detail.

This work aims at helping model users to better understand i) the model concepts used; ii) the differences between models and iii) the limits of the models. Seven published models have been chosen for this study: (1) ASM1 (Henze *et al.*, 1987; 2000a); (2) ASM2d (Henze *et al.*, 1999; 2000b); (3) ASM3 (Gujer *et al.*, 1999; 2000); (4) ASM3+BioP (Rieger *et al.*, 2001); (5) ASM2d+TUD (Meijer, 2004); (6) Barker&Dold model (Barker and Dold, 1997); (7) UCTPHO+ (Hu *et al.*, 2007). Nine standard processes have been identified and will be discussed in separate sections of this paper: hydrolysis; fermentation; ordinary heterotrophic organisms (OHO) growth; autotrophic nitrifying organisms (ANO) growth; OHO & ANO decay; poly-hydroxyalkanoates (PHA) storage; polyphosphate (polyP) storage; phosphorus accumulating organisms (PAO) growth and PAO decay.

For each standard process, (i) a brief overview on the available biochemical knowledge is provided as basis for discussion of the modelling concepts. The major publications are cited for further reading. Then, (ii) the different modelling concepts used are compared through a new schematic representation of the stoichiometry and the kinetics. Finally, (iii) the consequences of the model simplifications are investigated to draw theoretical limits of the models. Alternative published models that address the studied model limits are cited.

The final discussion synthesises the modelling concept diversity and the grey areas in theoretical knowledge, and discusses the model selection and existing model modifications.

2. METHODOLOGY

2.1. Studied models

The seven published models have been chosen among those most commonly reported in literature (Table 1). To keep the paper readable, those references will not be repeated each time. Two of the seven models, ASM1 and ASM3, only consider carbon and nitrogen removal, whereas the others also consider biological phosphorus removal. UCTPHO+ is an update of the UCTPHO model (Wentzel *et al.*, 1992), and ASM2d+TUD is the last published version of the integrated ASM-TUD (Technical University of Delft) metabolic model. This paper is using the corrected model formulations for typos and continuity problems (Hauduc *et al.*, 2010b). The full Gujer Matrices are available in a spreadsheet format as additional material through that reference.

Table 1 indicates the size of the models through the number of processes, state variables, interacting processes and variables (number of non-empty cells of the stoichiometric matrix) and parameters they include. It can be deduced that differences come mainly from the PAO processes. However, the real difficulty for model users results from the number of parameters

that should actually be calibrated (Hauduc *et al.*, 2010a). Many parameters, particularly stoichiometric, seldom require adjustment. Some kinetic parameters (e.g. hydrolysis and decay processes) are routinely calibrated in practice. Understanding the different modelling approaches and their concepts greatly aids in determining when a parameter should be calibrated for a given situation.

2.2. Model processes

Nine standard processes have been identified and are listed in Table 2. These "standard processes" involve mechanisms that only differ by the environmental conditions under which they take place. For instance, aerobic and anoxic OHO growth processes are combined as one OHO standard growth process.

Table 2 also synthesises the processes considered in each model. This work is limited to biological processes, and therefore chemical phosphorus precipitation is not discussed.

As OHO- and ANO-related processes of ASM2d+TUD are exactly the same as ASM2d, ASM2d+TUD will be studied only for BioP-related processes.

2.3. A new schematic representation

A new schematic representation of the model processes is proposed to facilitate model concept comparison in a systematic and transparent way. For each process type, the standard processes that use the same modelling concept are represented on a single figure, and the standard processes that are different in terms of modelling concept are represented on separate figures. The process is represented as a reaction with consumed components on the left of the figure and produced components on the right. Figure 1 shows the symbols used for the schematic representation:

- Models that consider the process are given by a letter (a to g).
- The electron acceptor condition of the process is indicated by a square (Ox: aerobic; Ax: anoxic; An: anaerobic), close to the corresponding model name.
- The included state variables are represented through both a shape and a background: the shape indicates whether the variable is particulate or colloidal, soluble or refers to an organism and internal storage, and the background indicates its composition in terms of ThOD (theoretical oxygen demand) (C), nitrogen (N) or phosphorus (P). The state variable name is indicated inside the shape, using the standardised notation from Corominas *et al.* (2010).
- The electron acceptor consumed in the process is represented by a square. For instance, depending on its usage, nitrate can be represented by a circle-square (electron acceptor) or by a circle only (substrate).

To simplify the graphs, alkalinity and total suspended solids are not represented. Only important stoichiometric coefficients (especially yields) are specified, as others can be calculated through conservation of ThOD, nitrogen and phosphorus (Hauduc *et al.*, 2010b).

In this process representation, the consumed and produced components of the reaction are linked by black arrows. In case state variables are not considered by all models or under all conditions, the models or conditions concerned are specified on the arrow.

The kinetic expression for the rate of reaction is also part of the concept and is therefore represented. This is also done in a condensed way by a standardised compact notation. Table 3 illustrates the different symbols used to keep the expression readable. The saturation functions and inhibition functions, which have the form of a Monod expression, are expressed $M()$ or $IM()$, with the component concerned in parenthesis. The symbol $\langle \rangle$ is used to indicate

optional or alternative terms depending on the model or the environmental condition (see Table 3 for examples).

2.4. Literature review on theoretical knowledge vs modelling concepts

The results are organised around the nine main processes listed in Table 2. For each process, the theoretical knowledge is first presented based on a brief literature review. Then the different concepts used in the models are analysed, supported by the schematic representation of the process. Different concepts are given different numbers (concept 1, concept 2...), whereas variations within the same concept are pointed out using letters (concept 1a, 1b...). Finally, model limitations in using particular concepts are listed.

3. RESULTS

3.1. General modelling concepts

3.1.1. State variables

Some conceptual differences among the studied models come from the state variables used, as summarised in Table 4.

All studied models are theoretical COD (ThOD) based. ThOD is the chosen organic material measure because it is a conservative quantity that allows characterising the electron equivalents of organic substrates, biomass and electron acceptors (Ekama and Marais, 1979; Henze *et al.*, 2000a). However a discrepancy exists between ThOD and analytical COD for compounds with negative ThOD, such as nitrite which is analytically measured as positive COD (Gujer and Larsen, 1995), moreover some compounds are not detected in a standard COD test.

3.1.2. *Component-based / fraction-based models*

In the ASM1 and the Barker & Dold model, organic nitrogen is considered in separate state variables (component-based model) and organic phosphorus is not considered. In these models nitrogen and phosphorus (Barker & Dold only) are, however, linked to biomass. In other models, nitrogen and phosphorus are linked to ThOD state variables (fraction-based models).

3.1.2.1. Component-based model

Pros. As variables are separated, the hydrolysis processes of organic ThOD and organic nitrogen are independent. It is thus easier to change parameters in case of variations in influent fractions.

Cons. In the Barker & Dold model, organic phosphorus is not considered separately. Phosphorus is then always available in the form of PO_4^{3-} , without any delay due to hydrolysis, when released by biomass decay.

3.1.2.2. Fraction-based model

Pros. Having linked variables limits the number of variables and processes.

Cons. The substrate fraction is supposed to be homogeneous and constant in its composition. In reality different organic compounds with different fractions are coming in the influent. In this case this concept could induce a pitfall or the model complexity must be increased by considering more components.

3.1.3. *Undegradable organics from influent and from biomass decay*

ASM1, the Barker & Dold model and UCTPHO+ distinguish the influent undegradable organics, from those formed by biomass decay (or endogenous respiration). The latter fraction thus includes non-active biomass. This distinction allows a different nitrogen and phosphorus

fraction content in soluble undegradable matter from influent and endogenous products, especially for the component-based models (ASM1 and Barker & Dold model, see above), increasing model flexibility.

3.1.4. N_2 considered as a state variable to close the nitrogen balance

To simplify the models, ASM1, the Barker & Dold model and UCTPHO+ do not consider dissolved dinitrogen gas (N_2) as a state variable. As a consequence, the continuity is not verified for these processes in terms of nitrogen (Hauduc *et al.*, 2010b). This state variable needs to be added to check the continuity of the model.

3.1.5. Total suspended solids (TSS) as a state variable

TSS is a combined variable in some models and a state variable in others, calculated from the linear combination of particulate state variables and from an assumed VSS/COD and VSS/TSS ratio (VSS being the volatile suspended solids) to predict the sludge mass in the system. Consequently, the ISS (inorganic suspended solids), which are defined as part of the TSS together with the VSS ($TSS = VSS + ISS$), are only empirically predicted in these models through the ratio VSS/TSS, which leads to unreliable estimations of TSS. Ekama and Wentzel (2004) propose a model for ISS that takes into account ISS from the influent, the precipitation of minerals and the inorganic content of biomasses.

TSS is not considered in this study.

3.1.6. Alkalinity

A low alkalinity value ($< 50 \text{ g CaCO}_3 \cdot \text{m}^{-3} = 1 \text{ mol HCO}_3^- \cdot \text{m}^{-3}$) results in an unstable pH, which could cause nitrification inhibition and other process problems (Henze *et al.*, 2000a). Alkalinity is thus modelled to predict the risk of pH limitation. As discussed previously

(Hauduc *et al.*, 2010b), alkalinity is considered or not depending on the models. In this study, alkalinity is not considered.

3.2. OHO and ANO processes

3.2.1. Hydrolysis of particulate substrate

3.2.1.1. Knowledge

A large fraction of wastewater substrate is particulate or colloidal, and is thus not directly available for biomass growth (Ekama and Marais, 1979; Morgenroth *et al.*, 2002). Hydrolysis is an extracellular biological reaction where hydrolytic enzymes break down large organic molecules into smaller ones that can pass through the bacterial cell wall. Hydrolytic enzymes seem to be bound to the floc and have a low turnover rate (hours to days) which enable hydrolysis to be decoupled from the enzyme synthesis (Goel *et al.*, 1999).

Substrate. The diversity of substrates, hydrolytic enzymes and biological pathways make the hydrolysis process difficult to study. Experiments described in literature are mainly based on pure culture bacteria, with single or few substrates, providing results that can hardly be generalised.

Protozoa are also able to take up particulate substrate as shown by de Kreuk *et al.* (2010), and possibly release readily biodegradable substrate in the process. However, this process is so far poorly described.

Electron acceptor conditions. Hydrolytic enzyme synthesis depends on the electron acceptor conditions, but their activity is not affected by the electron acceptor conditions (Goel *et al.*, 1999), which enables hydrolysis processes to continue under anoxic and anaerobic conditions. Hydrolysis due to protozoa activity will however depend on oxic conditions.

3.2.1.2. Modelling

In the models studied here, only two concepts are used (Table 5):

- The first concept is one step hydrolysis, where slowly biodegradable substrate is hydrolysed, then consumed by organisms. The differences between the models concern the way the residues of the reaction and the nitrogen fractions are modelled (3.1.2):
 - Component-based model (ASM1, Barker & Dold);
 - Fraction-based model (ASM2d, ASM3, ASM3+BioP).
- The second concept used in UCTPHO+, is based on direct growth using adsorbed substrate. The hydrolysis is accounted for by a reduced growth rate for the use of this adsorbed substrate (see OHO growth process, Table 7). This way to model hydrolysis makes the hydrolysed substrate available for the organisms that produce hydrolysis enzymes, whereas in other modelling concepts the hydrolysed substrate is released into the bulk phase, in this way becoming available for all organisms, which will thus compete for it.

The hydrolysis process is in fact used to model all mechanisms that make slowly biodegradable substrate available for bacterial growth with a certain delay (chemical dissolution, mass transport, storage, etc.). Consequently, depending on the other processes considered in the model, the hydrolysis process does not have the same significance:

- Storage is considered as a separate process in ASM3 and ASM3+BioP, whereas it is not explicitly described in other models. However, storage and hydrolysis cannot be distinguished through respirometric methods (Goel *et al.*, 1999). Consequently, in ASM1, ASM2d, Barker & Dold and ASM2d+TUD, the storage is implicitly included in the hydrolysis process.

- Depending on the origin of the organic molecules, two types of hydrolysis reactions can be distinguished: hydrolysis of "primary substrate" that comes from the influent and hydrolysis of the matter produced by biomass metabolism or decay, named "secondary substrate", in which protozoa may play an important role (Morgenroth *et al.*, 2002). Consequently, models using the death-regeneration concept to model biomass decay (see paragraph 3.2.5.2) merge those two types of hydrolysis in a single process, whereas in case of the endogenous respiration concept, the hydrolysis of secondary substrate is modelled through endogenous respiration and maintenance processes (ASM3 and ASM3+BioP).

Electron acceptor conditions. The storage process and the utilisation of secondary substrate require an electron acceptor to produce energy. Models that implicitly merge these processes into the hydrolysis process (ASM1, ASM2d, Barker & Dold model, UCTPHO+ and ASM2d+TUD, see above), have then to take into account their electron acceptor. Thus, the hydrolysis kinetic rates should depend on the electron acceptor. However, ASM1 does not consider hydrolysis under anaerobic conditions. As ASM3 and ASM3+BioP consider storage and hydrolysis separately, the electron acceptor is not rate limiting for hydrolysis.

The Barker & Dold model introduces a hydrolysis yield in anoxic and anaerobic conditions that allows modelling the experimentally observed "COD loss" (Barker and Dold, 1995). Although this observation is not explained so far, the "loss" is modelled by H₂ gas formation (Kraemer *et al.*, 2008). The S_{H2} state variable is thus added to the model to reach continuity (Hauduc *et al.*, 2010b).

As UCTPHO+ models the hydrolysis process simultaneously with growth, anaerobic hydrolysis is not modelled.

Ammonification. In case of component-based models (ASM1, Barker & Dold), biodegradable organic nitrogen is produced by the hydrolysis process. To make this nitrogen available for organisms, ammonification has to be modelled.

3.2.1.3. Model limitations

Substrate. The concept of one step hydrolysis is used by all models but one (UCTPHO+). This concept implies a simplification of the (primary) substrate into a single biodegradable particulate fraction. In case of peculiar influents with different particulate substrates behaviour or large colloidal fractions, it may be required to integrate other particulate fractions and to consider other hydrolysis concepts, such as parallel hydrolysis or sequential hydrolysis (Orhon *et al.*, 1998; Nowak *et al.*, 1999; Larrea *et al.*, 2002).

Electron acceptor conditions. Hydrolysis enzyme activity is independent of the electron acceptor (Goel *et al.*, 1999). However, the hydrolysis process also covers other mechanisms that require an electron acceptor, such as degradation by protozoa and storage. In case of a large anaerobic zone, anaerobic hydrolysis should be considered, especially for BioP models to make substrate available for PHA storage (ASM1 and UCTPHO+).

Experimental determination of parameters. Modelling hydrolysis and storage as two separated processes as in ASM3 and ASM3+BioP, requires adequate experiments to independently determine the kinetic rates (Goel *et al.*, 1999).

3.2.2. Fermentation

3.2.2.1. Knowledge

Fermentation is a growth process under anaerobic conditions for OHOs. In the absence of an electron acceptor, oxidative processes inside the cells are not possible and the substrate is

partially oxidised to CO_2 and partially reduced to products. Under these conditions, organic substrate is catabolised into volatile fatty acids (VFA, e.g. acetate), associated to organisms growth.

3.2.2.2. Modelling

Two different concepts are used to model fermentation in the studied models (Table 6):

- The first concept considers fermentation as a transformation (ASM2d, UCTPHO+)
- In the second concept fermentation is described as an anaerobic growth process.

The process kinetic rate always depends on the OHO concentration, which is considered as the only biomass involved in this process.

Barker and Dold (1995) experimentally observed a COD "loss" during anaerobic processes, which they linked to fermentation, anaerobic hydrolysis and S_{VFA} sequestration. This phenomenon has been modelled by a S_{VFA} formation yield (Y_{fe}) in the fermentation process. The loss of $(1-Y_{\text{fe}})$ g ThOD.g S_{VFA}^{-1} is modelled through H_2 gas formation by Kraemer *et al.* (2008), and a S_{H_2} state variable has thus been added to reach model continuity (Hauduc *et al.*, 2010b).

3.2.2.3. Model limitations

ASM1 and ASM3 do not consider fermentation, consequently only one soluble substrate is considered (S_{B}). Fermentation is considered only in models with BioP, since PAOs are assumed to only grow on fermentation products (S_{VFA}) and not on fermentable products (S_{F}). However, fermentation is not considered in ASM3+BioP: hydrolysis is considered as the rate-limiting step, so that the fermentation process rate does not need to be considered explicitly. This could be a model limitation in cases where hydrolysis is no longer the rate-limiting step,

e.g. in the case of a peculiar influent (e.g. from agro-industries with high S_B concentration), or a specific plant configuration (e.g. with hydrolysis of return activated sludge).

All models except the Barker & Dold model neglect OHO formation during fermentation. Indeed, Ekama and Wentzel (1999) estimate the anaerobic growth yield at $0.10 \text{ g } X_{\text{OHO}} \cdot \text{g } S_B^{-1}$. In the case of large anaerobic zones, anaerobic growth may not be neglected and concept 2 (Table 6) should be chosen.

3.2.3. Ordinary heterotrophic organisms (OHO) growth

3.2.3.1. Knowledge

Under aerobic or anoxic conditions OHOs use organic substrate as an energy and carbon source. The yield of biomass growth is the fraction of substrate that is used as a carbon source to produce biomass (figure 2).

Substrate. van Loosdrecht *et al.* (1997b) proposed the existence of two types of bacteria in terms of their capacity for substrate storage. Bacteria not capable of substrate storage will maximize their growth rate in periods with available substrate in order to be competitive, but will not be able to maintain their cell structure in case of long starvation periods. In case of a highly dynamic influent or in case of a process with a feast/famine cycle, bacteria capable of storing substrate will have a strong competitive advantage due to their ability to maintain a low growth rate during starvation periods, which enables them to keep all of their cell system viable (van Loosdrecht *et al.*, 1997b).

Stored compounds, e.g. poly- β -hydroxybutyrate (PHB), result from additional substrate that is taken up on top of the substrate requirement for direct growth (van Aalst-Van Leeuwen *et al.*, 1997). However, the nature of the storage compounds is still not well understood but seems to

depend on the substrate used (Beccari *et al.*, 2002). For instance, glycogen can be formed from glucose (Dircks *et al.*, 2001), and cases where propionate constitutes an important substrate fraction in the influent would lead preferentially to an increased poly- β -hydroxyvalerate (PHV) fraction as compared to PHB (Oehmen *et al.*, 2007).

The substrate uptake rate increases instantaneously when a high substrate concentration occurs (up to the maximum rate), but the growth rate increases only slowly, and the extra substrate taken up may be stored (van Aalst-Van Leeuwen *et al.*, 1997). Consequently, a high growth rate (e.g. at short SRT) will result in less storage (Beun *et al.*, 2002; van Loosdrecht and Heijnen, 2002). The growth rate on stored compounds is lower and limited by the storage product degradation process, which depends on its content of the biomass following a first order relationship (van Loosdrecht *et al.*, 1997b; Beun *et al.*, 2002).

Nutrients. Organism growth also requires nutrients such as nitrogen or phosphorus to synthesise their cells constituents (proteins, nucleic acids...). In case of ammonia depletion, OHOs are able to use nitrate as nitrogen source. For instance, Wentzel *et al.* (1989) experimentally proved that when ammonia is depleted, PAOs consume nitrates for their growth with no modification of their kinetic behaviour. However, the yield will be slightly lower since some ThOD is used to reduce nitrate to ammonium.

Denitrification. Heterotrophic growth under anoxic conditions requires oxidised forms of nitrogen as electron acceptor: nitrate (NO_3^-), nitrite (NO_2^-), nitric oxide (NO) or nitrous oxide (N_2O). If denitrification is complete, these electron acceptors are reduced sequentially to nitrogen gas (N_2).

The need to use a different OHO yield under anoxic conditions ($Y_{\text{OHO,Ax}}$) to properly model the nitrate and COD consumptions was experimentally shown by several authors (Orhon *et al.*, 1996; Sozen *et al.*, 1998; Muller *et al.*, 2003). This is due to the lower ATP (adenosine

triphosphate, which transports energy in the cell) formation through oxidative phosphorylation in the electron transport chain (lower ATP formation per NADH ($Y_{\text{NADH_ATP}}$) in metabolic models) when nitrate is the electron acceptor instead of oxygen (Beun *et al.*, 2002; Muller *et al.*, 2003).

3.2.3.2. Modelling

The stoichiometry of OHO growth requires an organic substrate, an electron acceptor and nutrients. The modelling concepts differ in the substrates used, the nitrogen source and the use of different yields for aerobic and anoxic conditions (Table 7):

- The first concept considers direct growth of OHOs on readily biodegradable substrate:
 - In concept 1a, NH_x is the only nitrogen source (ASM1, ASM2d),
 - In concept 1b, NO_x can be used as a nitrogen source in case of ammonia depletion (Barker & Dold, UCTPHO+). Additionally, UCTPHO+ considers adsorption of particulate substrate onto OHOs, followed by direct growth on the adsorbed substrate.
- The second concept first considers substrate storage and then OHOs growth on storage compounds as the unique carbon source (ASM3, ASM3+BioP).

The adsorption and storage processes are particularly useful in case of cyclic loading conditions and selector modelling. The kinetics of these processes are considered to depend on the ratio of adsorbed or stored substrate to biomass and are associated with a maximum adsorption/storage potential (Ekama and Marais, 1979). The kinetic expression for adsorption in the UCTPHO+ model is in agreement with this statement. However, ASM3 and ASM3+BioP only use a Monod expression for substrate uptake, and thus consider that the maximum storage potential cannot be reached under normal wastewater treatment conditions.

Substrate: Several substrates are used depending on the models (Table 8):

- Readily biodegradable substrate (S_B) or fermentable substrate (S_F) are used by all models except ASM3 and ASM3+BioP, which model indirect growth only.
- Volatile fatty acids (S_{VFA}) are considered in Bio-P models, except ASM3+BioP. For this substrate, OHOs compete with PAOs when present under aerobic or anoxic conditions.
- Adsorbed particulate substrate ($X_{OHO,Ads}$) is considered in UCTPHO+. This substrate has to be hydrolysed before use, which occurs simultaneously with growth. Modelling adsorption processes is a way to slow down OHO substrate consumption and model the delay observed before growth occurs under certain conditions (feed/starvation). This way to model hydrolysis is chosen by the UCT group to avoid the competition of organisms on hydrolysed substrate with the hydrolysis products being consumed directly by the organisms that produce hydrolysis enzymes (Wentzel *et al.*, 1992).
- Stored substrate ($X_{OHO,Stor}$) is the only usable substrate in ASM3 and ASM3+BioP. Direct growth on external substrate is not considered. This concept is needed in alternating feeding/starvation phases of the plant; mainly for selector systems. It allows simulating the observed delay before OHO growth.

A substrate preference switching function should be used to avoid that the OHO specific growth rate increases above a maximum value if two substrates are present in high concentration (Henze *et al.*, 2000b) as two OHO growth processes run in parallel. The substrate preference switching function usually used in ASM models is in the form:

$$\left(\frac{S_{Sub}}{K_{SSub} + S_{Sub}} \right) \cdot \frac{S_{Sub}}{\sum_i S_{Sub,i}}$$

with S_{Sub} being the considered substrate.

Nutrients. Barker & Dold and UCTPHO+ consider growth with NO_3 as nitrogen source in case of ammonia depletion. However, these models do not consider the reduction of nitrate in the redox balance.

Denitrification. Denitrification is modelled as one step: nitrate is considered the only possible electron acceptor. The maximum anoxic growth rate is lower than under aerobic conditions, either because $\mu_{\text{OHO,Max}}$ is intrinsically lower for OHOs under anoxic conditions, or because only a fraction of OHOs is able to denitrify. Furthermore, all models but two (ASM1 and ASM2d) use a lower anoxic growth yield (Table 8), since the efficiency of oxidative phosphorylation is lower under anoxic conditions.

3.2.3.3. Model limitations

Adsorption and storage. The ASM3 growth on stored substrate does not consider direct growth on soluble substrate. This might lead to inaccurate predictions in case of low SRT (<5 d) (van Loosdrecht and Heijnen, 2002), and long feast/famine cycles, which are conditions when growth rate and storage are not constant. Krishna and van Loosdrecht (1999), Karahan-Gül *et al.* (2003), Sin *et al.* (2005) and Guisasola *et al.* (2005) proposed ASM3 modifications considering parallel direct growth on soluble substrate and indirect growth on internally stored substrate.

Beccari *et al.* (2002) proposed a different modelling concept that includes first a biosorption step, in which substrate is absorbed by biomass without any transformation, contrary to the UCTPHO+ concept where substrate is adsorbed on the biomass. Then, the biosorbed substrate is used either for direct growth or is transformed into a stored compound, which is later used for growth. This modelling concept allows a better description of the ammonia profile,

because biosorption does not release the nitrogen content of the substrate into the mixed liquor, contrary to external hydrolysis of the adsorbed compound.

Denitrification. In case of a large anoxic zone, using a single growth yield value for anoxic and aerobic processes (ASM1, ASM2d) could lead to an overestimation of the denitrification process in terms of biomass production, and underestimation of substrate consumption and nitrogen removal. The underprediction of substrate consumption could also have an effect on other processes such as P removal. A different anoxic growth yield should be added, but the model will then require a recalibration of the hydrolysis and storage processes to compensate the substrate consumption and maintain the experimentally observed denitrification rate (Muller *et al.*, 2003).

3.2.4. Autotrophic Nitrifying Organisms (ANO) growth

3.2.4.1. Knowledge

ANO oxidise ammonia to produce the required energy for CO₂ uptake and growth. This oxidation of ammonia is named nitrification. It includes two steps that involve two distinct groups of autotrophic organisms: ammonia oxidisers and nitrite oxidisers (figure 3). In the first step, nitrification, ammonia oxidisers produce energy required for their growth through ammonia oxidation into nitrite. Then, in the nitrification step, nitrite oxidisers convert nitrite into nitrate to produce energy. The first oxidation (nitrification) consumes alkalinity (Downing *et al.*, 1964).

Because a lot of energy is required to reduce CO₂, autotrophic organisms have a lower growth yield than OHOs. Furthermore, the nitrifiers, which are obligate aerobic organisms, have a higher requirement of oxygen than heterotrophs for their growth: in addition to their needs in electron acceptor for respiration, oxygen is used to oxidise ammonia. Therefore, to ensure

good nitrification, it is necessary to provide sufficient dissolved oxygen to the activated sludge and to maintain a minimum SRT to avoid the wash out of nitrifiers (Downing *et al.*, 1964). Nitrification is also inhibited by a low pH and sufficient alkalinity concentration (generally $>50 \text{ g CaCO}_3 \cdot \text{m}^{-3} = 1 \text{ mol HCO}_3^- \cdot \text{m}^{-3}$) has thus to be maintained to ensure a stable pH (Henze *et al.*, 2000a).

3.2.4.2. Modelling

Nitritation is normally considered the limiting step in nitrification (Downing *et al.*, 1964). Consequently, nitrification is often modelled as a one step process, as in all studied models (Table 9), and initially proposed by Lijklema (1973).

3.2.4.3. Model limitations

Multi-step nitrification/denitrification. The simplified concept of one-step nitrification is sufficient for most municipal wastewater systems.

However, the modelling project may require predicting nitrite accumulation (shortcut nitrification-denitrification, inhibitions...) or greenhouse gas emission, in the form of nitric and nitrous oxide. NO_2^- accumulation (partial nitrification) has actually been observed in specific situations such as unstable operation of municipal WWTP (e.g. due to insufficient oxygen, low temperature, low sludge age and inhibitory compounds), high temperatures, side stream processes or industrial influent (Sin *et al.*, 2008; Kaelin *et al.*, 2009).

These modelling objectives cannot be reached with any of the studied models, which consider nitrification and denitrification as a single step. Consequently some models have been extended with two step nitrification and denitrification, as reviewed by Sin *et al.* (2008). A model with four step denitrification (NO_2 , NO and N_2O as intermediates) and two step

nitrification is also proposed by Hiatt and Grady (2008). Currently, considerable attention is paid to greenhouse gas production in wastewater treatment and in the near future this will certainly lead to much more detailed models of the nitrogen-related reactions.

Nitrification inhibition. Autotrophs are sensitive to inhibition (pH, nitrous acid, ammonia, chromium, nickel, copper, etc). Effects of some environmental conditions on the activated sludge process are reported in Gujer (2010). Inhibitory effects are considered to be constant and are thus accounted for in the growth rate value (Henze *et al.*, 2000a). This can cause calibration problems in case of variability in the concentration of these compounds in the influent or in the treatment plant. To detect variability in influent inhibitors, some authors developed online respirometric methods to determine inhibition kinetics of nitrification (Nowak *et al.*, 1995; Kong *et al.*, 1996; Vanrolleghem *et al.*, 1996).

3.2.5. *OHO and ANO decay*

3.2.5.1. Knowledge

van Loosdrecht and Henze (1999) published a literature review on the theoretical knowledge regarding maintenance, endogenous respiration, lysis, decay and predation. Oxygen consumption linked to a loss of biomass was observed by various authors since the end of the 19th century. This phenomenon has been explained by the concept of "endogenous respiration" during which bacteria use their own storage pools of organic matter for maintenance purposes instead of using external substrate. Other experiments have shown accumulation of undegradable matter in the absence of substrate, leading to the cryptic growth (growth on dead bacteria) or the "death-regeneration" concepts (van Loosdrecht and Henze, 1999).

These concepts lump several mechanisms that result in oxygen consumption and biomass reduction (van Loosdrecht and Henze, 1999; Hao *et al.*, 2010):

- dormancy of bacteria: non-active state, which is seen as undegradable particulate.
- internal decay: consumption of internally stored compounds in case of starvation. This mechanism is not coupled to significant microorganism death.
- external decay: predation by protozoa, viral attack, and cell lysis (phages, etc). The cell walls may not be degraded by protozoa and phages, which results in unbiodegradable material production.
- maintenance: the energy required to maintain essential life conditions in the cell, such as cell motility, maintenance of ion gradients, turnover of cell material (e.g. proteins, RNA) and transport of material (Lopez *et al.*, 2006; Hao *et al.*, 2010).

It should be noted that the electron acceptor conditions have been found to influence the OHO and ANO decay rates. The nitrification activity has been shown to be higher after prolonged exposure to anaerobic and anoxic conditions as compared to aerobic conditions (Siegrist *et al.*, 1999; Munz *et al.*, 2011), where the ANO decay rate was >50% lower under anaerobic and anoxic conditions. A reduced anoxic vs aerobic decay rate was also observed for OHOs (Siegrist *et al.*, 1999).

3.2.5.2. Modelling

Two concepts are used (Table 10):

- The death-regeneration concept. Two sub-concepts have to be distinguished:
 - Death-regeneration with a component-based model (ASM1, Barker & Dold);
 - Death-regeneration with a fraction-based model (ASM2d, UCTPHO+).
- The endogenous respiration concept (ASM3, ASM3+BioP).

For OHOs and ANOs, maintenance is considered as negligible and is considered part of the decay or endogenous processes in all models.

Death-regeneration concept. The biomass decay results in the release of a fraction $(1 - f_{XU_OHO,lys})$ of particulate substrate and a fraction $f_{XU_OHO,lys}$ of undegradable material. The released particulate substrate will be hydrolysed, and then used again for OHO growth. Consequently, ANO decay contributes to OHO growth.

This concept also allows modelling anaerobic decay, and modelling the high oxygen or nitrate demand observed after an anaerobic condition period (Warner *et al.*, 1986), which would not be possible with the endogenous respiration concept. However, maintenance and endogenous respiration are neglected.

Endogenous respiration concept. This concept is closer to experimental observations (Gujer *et al.*, 2000). In this process energy is provided by the oxidation of the organic matter contained in biomass, which leads to undegradable matter and nutrients release. As a consequence, there is no cycling of ThOD in the model, which simplifies model calibration. Models that consider a storage pool (ASM3, ASM3+BioP) have to consider storage degradation for maintenance: stored compounds are used to produce energy without biomass production. This process is similar to the maintenance concept of PAOs (see 3.3.4.2), and explains the fate of the OHO storage pool during OHO decay. This can be considered as endogenous respiration of the storage pool.

3.2.5.3. Model limitations

Biokinetic models using the endogenous respiration concept should have better identifiable parameters and should thus be easier to calibrate (Gernaey *et al.*, 2004). Indeed, the endogenous respiration concept parameters only influence the decay process of the considered

organism, whereas the death-regeneration concept parameters influence the decay of the concerned organism (autotrophs and heterotrophs), hydrolysis and the growth processes of heterotrophs (substrate availability). Furthermore, the death-regeneration concept induces a higher biomass production rate, which has a general effect on all kinetic rate constants. Consequently, kinetic parameters are not directly comparable between models using the endogenous respiration concept or the corresponding death-regeneration concept as presented by Dold *et al.* (1980).

Predation. Predation is explicitly modelled by Curds (1971), Lijklema (1973), Moussa *et al.* (2005) and more recently by Ni *et al.* (2010), considering a reduction of the active biomass through protozoa consumption, their concentration being between 5% and 10% of the MLVSS according to Curds (1971). Not considering predation may lead to variable kinetic parameter values depending on the WWTP conditions.

Electron acceptor conditions. The concept of endogenous respiration does not allow decay under anaerobic conditions, since no electron acceptor for the respiratory chain is available. The death-regeneration concept has been developed to cope with the anaerobic decay process in case the endogenous respiration concept is used and to keep the model as simple as possible (Dold *et al.*, 1980). However, under anaerobic/anoxic conditions predation by protozoa does not occur since they are strictly aerobes, and ANO and OHO decay rates have been shown to be lower (Siegrist *et al.*, 1999). Consequently, anaerobic/anoxic decay could be considered as negligible under certain WWTP conditions. Alternatively, these lower anaerobic and anoxic decay rates could cause an underprediction of biomass concentrations; especially in cases of long periods with unsuitable nitrification conditions (rain events, weekends, holidays, etc.) (Siegrist *et al.*, 1999).

3.3. Biological phosphorus removal

Phosphorus accumulating organisms (PAOs) have the ability to store carbon compounds in excess of normal metabolic requirements as poly- β -hydroxyalkanoates (PHA) and glycogen, and to store phosphorus in the form of polyphosphate (polyP). This ability is used in wastewater treatment to biologically remove phosphorus, by stimulating PAO growth by a sequence of anaerobic and aerobic (or anoxic) conditions. PAO metabolism is usually described by 2 or 3 steps:

- substrate uptake (usually volatile fatty acids, S_{VFA}) and storage as PHA, typically under anaerobic conditions, associated with glycogen (ASM2D+TUD) and polyP consumption (all bio-P models);
- PolyP and glycogen storage pools restoration and PHA consumption under aerobic and anoxic conditions (modelled simultaneously with growth in the Barker & Dold and UCTPHO+ models);
- PAO growth associated to PHA consumption under aerobic and anoxic conditions.

Organic substrate uptake under anaerobic conditions provides PAOs a competitive advantage over OHOs. Furthermore, the anaerobic conditions enable the formation of S_{VFA} from fermentable substrate S_F . The simplified mechanisms of these steps are represented in Table 11. The use of PAOs to biologically remove phosphorus is named the enhanced biological phosphorus removal process (EBPR or BioP).

Metabolic model. To conceptualise BioP, the Delft University of Technology (TUD) group introduced a metabolic model that considers cell internal reactions (Smolders *et al.*, 1994a; Smolders *et al.*, 1994b). The cell internal concentrations of metabolites (NADH, acetyl-CoA, ATP, etc.) are considered to be in steady state conditions. Consequently, these components

are not modelled, and only the overall stoichiometric reaction is formulated. This results in a model structure that is similar to the others.

3.3.1. PHA storage

3.3.1.1. Knowledge

Under anaerobic conditions, in the presence of substrate, PAOs store PHA. Figure 4 illustrates the main biochemical steps of PHA storage. Some experiments (Comeau *et al.*, 1987; Wentzel *et al.*, 1989; Brdjanovic *et al.*, 1998a) indicated that PAOs can also store PHA under anoxic or even aerobic conditions, if sufficient substrate is available.

Energy source. VFAs are transported in the undissociated form (associated to a proton), which causes dissipation of the membrane proton motive force. PolyP breakdown and phosphate release associated to proton ions allow the re-establishment of the proton motive force (Comeau *et al.*, 1986). This phosphate release is also concomitant with Mg^{2+} and K^{+} release, which serve as counter-ions for stabilisation of the polyP chain (Wentzel *et al.*, 1986).

The polyP breakdown also provides most of the required energy to metabolise substrate into acetyl-CoA (Comeau *et al.*, 1986) by phosphorylation of AMP into ADP (and later to ATP) through the AMP-phosphotransferase enzyme (Wentzel *et al.*, 1992).

Reducing power. Two theories for NADH production were developed: the "Comeau-Wentzel model" and the "Mino model" (Jenkins and Tandoi, 1991; Wentzel *et al.*, 1992).

- The "Comeau-Wentzel model" hypothesises that NADH is provided by the anaerobic oxidation of acetate through the TCA cycle.
- The "Mino model" considers that the NADH is provided by glycolysis under anaerobic conditions, turning stored glycogen into pyruvate and then into acetyl-CoA

645 and CO₂ (Mino *et al.*, 1998; Oehmen *et al.*, 2007). This reaction also provides energy
646 for acetate uptake and conversion to acetyl-CoA.

647 The Mino model theory is well accepted and supported by experimental evidence, but the
648 oxidative part of the TCA cycle seems to effectively supply part of the reducing power for
649 PHA formation under certain conditions (Mino *et al.*, 1998; Zhou *et al.*, 2010). Oehmen *et al.*,
650 (2007) hypothesise that either each metabolic pathway is used by a specific microbial group
651 of PAOs, or that PAOs are able to use different metabolic pathways depending on their
652 internal or external conditions.

653 PolyP storage pool. Mino *et al.* (1985) and Wentzel *et al.* (1989) observed that not all the
654 stored polyP can be degraded. They hypothesised that two different polyP molecular weights
655 exist: short polyP chains have low molecular weight and can be released, whereas long polyP
656 chains cannot. However, no experimental evidence has supported this hypothesis so far.
657 Glycogen limitation, however, has been shown to result in the incomplete degradation of
658 polyP (Brdjanovic *et al.*, 1998c). Such a limitation may occur at a low pH (less than 7.3) in
659 the presence of an excess of VFAs.

660 Substrate. Other substrates than acetate can be used by PAOs (Oehmen *et al.*, 2007) such as
661 carboxylic acids, sugars and amino acids (Mino *et al.*, 1998). However, most experiments
662 have been carried out on enriched cultures with acetate, which is usually considered as the
663 unique substrate source in order to simplify the models (Mino *et al.*, 1998).

664 pH dependency. The energy requirements for S_{VFA} uptake have been observed to increase
665 with pH, leading to an increased phosphorus release to S_{VFA} uptake ratio. This is interpreted
666 as a higher energy required for maintaining the proton motive force for S_{VFA} transport (Mino
667 *et al.*, 1998).

Competition with GAOs. PAOs have to compete with GAOs (glycogen accumulating non-polyP organisms) for the VFAs under anaerobic conditions. Indeed, GAOs store acetate as PHA under anaerobic conditions without using polyP reserves. GAOs use this PHA as carbon and energy source for aerobic/anoxic growth and glycogen production. Their glycogen storage is used both as energy and reducing power source for anaerobic substrate uptake (Mino *et al.*, 1998). Therefore, GAOs have to store more glycogen than PAOs (Sudiana *et al.*, 1999).

This competition seems to highly depend on external factors such as carbon source, pH, temperature, sludge age, dissolved oxygen concentration and inhibitory compounds (Meijer, 2004; Oehmen *et al.*, 2007). Lopez-Vazquez *et al.* (2009) concluded that GAOs are favoured by higher temperatures and lower pH.

3.3.1.2. Modelling

The concepts vary in terms of substrate used (S_B or S_{VFA}) and in terms of source of energy (Table 12):

- In the first concept, energy for storage is provided by polyP breakdown, and reducing power production is not considered (ASM2d, UCTPHO+, Barker & Dold, ASM3+BioP).
- In the second concept that only concerns ASM2d+TUD, energy is provided by polyP breakdown and glycogen degradation, while reducing power is also generated through glycolysis.

PHA storage. In the first concept glycogen storage is not distinguished from PHA storage. Consequently, the storage pool for these models is named $X_{PAO,Stor}$ (Corominas *et al.*, 2010).

Energy source. In the Barker & Dold model an additional observed need of energy is recognised in the form of a PHA formation yield. This causes a "COD loss", hypothesised to be H_2 formation for mathematical modelling (see 3.2.2.2).

Reducing power. In the first concept the redox balance in the cell is neglected. In the second concept, NADH production comes from glycogen hydrolysis under anaerobic conditions; and under anoxic conditions NO_x utilisation as electron acceptor in the oxidative phosphorylation pathway stimulates the TCA cycle that produces NADH/FADH. The aerobic/anoxic stoichiometry of ASM2d+TUD is dependent on 3 metabolic yields: ATP formation per NADH (Y_{NADH_ATP}), biomass production per ATP ($Y_{ATP_X,Bio}$) and NADH requirement for PO_4 transport across the cell membrane (Y_{NADH_P}) (Smolders *et al.*, 1994a; Smolders *et al.*, 1994b).

Substrate. For all models except ASM3+BioP, S_{VFA} is the unique PAO substrate. For ASM3+BioP, S_B is used as unique substrate for both PAOs and OHOs. Indeed, fermentation is neglected since hydrolysis is considered to be the rate limiting step. PAOs are then in competition with OHOs for substrate uptake under aerobic and anoxic conditions.

PolyP storage pools. The Barker & Dold model considers two types of polyP: low and high molecular weight fractions. Only polyP with low molecular weight can be released during the PHA storage process.

pH dependency. In ASM2d+TUD, the stoichiometry of anaerobic acetate uptake is dependent on the energetic (ATP) requirement for acetate uptake across the cell membrane (Y_{ATP_PHA}), therefore the anaerobic yield for S_{VFA} uptake is a function of pH.

Kinetics. The kinetic rate expression for PHA storage does not depend on the electron acceptor in the first concept (energy from polyP only), but does in the second one.

The rate is limited by the polyP concentration in Barker & Dold, UCTPHO+ and ASM2d+TUD and by the polyP storage pool filling ratio for ASM2d and ASM3+BioP.

3.3.1.3. Model limitations

Reducing power. In cases in which glycogen is depleted, the substrate storage may stop (Brdjanovic *et al.*, 1998c), and models using the first concept that neglects glycogen storage would overpredict substrate storage. However, depending on the PAO sub-group or on their internal or external conditions, some PAOs would be able to use the TCA cycle for reducing power formation, without using glycogen storage (Zhou *et al.*, 2010). Further research is needed on this topic.

Substrate. In ASM3+BioP, S_B is used as substrate with the hypothesis that hydrolysis is the rate-limiting step. PHA storage will be overestimated should fermentation become the rate limiting step, because less substrate will be available for PAOs.

PolyP storage pool. The Barker & Dold model considers polyP with high molecular weight as state variable, based on the observation of Wentzel *et al.* (1989) and Mino *et al.* (1985) that a quantity of polyP always remains despite PHA storage being stopped. However, glycogen can also be limiting the substrate uptake process (Brdjanovic *et al.*, 1998c; Mino *et al.*, 1998). As glycogen was not considered by Mino *et al.* (1985), their observation might in fact be due to glycogen depletion.

Competition with GAOs. In some cases phosphorus removal deterioration has been reported. Those cases are often related to growth of GAOs (Mino *et al.*, 1998) which can be included in a comprehensive model, as recently done by Oehmen *et al.* (2010).

3.3.2. PolyP storage

3.3.2.1. Knowledge

In the presence of an electron acceptor and the absence of an available carbon source, PAOs will restore their polyP and glycogen storage pools, a metabolism that provides them an ecological advantage over OHOs (Mino *et al.*, 1998).

PAOs have a high affinity for PO_4 and are able to store up to 12% of their dry weight as polyP granules (against 1 to 3% of P content of OHO) (van Loosdrecht *et al.*, 1997a), also called volutins (Buchan, 1983). Their ability to store polyP makes the PAOs very efficient in terms of phosphorus removal.

Energy source. PHA oxidation allows the establishment of a proton motive force, which allows phosphorus uptake and ATP formation through the ATP-ase. ATP is then used to form polyphosphates (Comeau *et al.*, 1986).

Denitrification. PAOs are also capable of simultaneous denitrification and phosphorus uptake under anoxic conditions, using either their stored PHA, or if available, S_{VFA} . However, the phosphorus uptake efficiency is lower with nitrate as electron acceptor, and thus more stored carbon is consumed as compared to aerobic conditions (Barker and Dold, 1996).

Glycogen storage. Glycogen is formed from PHA oxidation under aerobic and anoxic conditions (Smolders *et al.*, 1994a; Mino *et al.*, 1998).

3.3.2.2. Modelling

Models differ in the source of energy for polyP storage and in the overall concept for energy utilisation (Table 13):

- the first concept considers growth and polyP storage processes independently (ASM2d and ASM3+BioP). Consequently, PHA oxidation is the result of phosphate uptake and growth.
- in the second concept storage pool restoration and growth are coupled (ASM2d+TUD). A part of the energy provided by PHA oxidation is allocated to each process.
- UCTPHO+ and the Barker & Dold model include the polyP storage process in an overall growth process (described in the PAO growth paragraph). This concept is consequently close to the second one.

Energy source. The polyP storage process is linked to the growth process as they both use the same source of energy. In concept 1, the polyP storage is considered independently of PAO growth. Conversely, in concept 2 (ASM2d+TUD) polyP and glycogen storage pools restoration are coupled to PAO growth. Therefore, energy production for polyP storage has been represented mathematically as PAO biomass oxidation (Meijer, 2004). However, this process is considered to take place in parallel with glycogen storage and PAO growth in the linear Herbert-Pirt relation for growth and product formation (Herbert *et al.*, 1956; Pirt, 1965):

$$q_{S,obs} = \frac{\mu_{obs}}{Y_{gro}} + \frac{q_{S_Prod,obs}}{Y_{Prod}} + m_S$$

where $q_{S,obs}$ is the observed specific substrate conversion rate (g COD.g COD⁻¹.h⁻¹), $\mu_{obs,S}$ the observed specific growth rate (h⁻¹), $q_{S_Prod,obs}$ is the observed specific product formation rate (g COD.g COD⁻¹.h⁻¹), Y_{gro} the growth yield (g COD.g COD⁻¹), Y_{Prod} the product yield (g COD.g COD⁻¹) and m_S the substrate specific maintenance rate (g COD.g COD⁻¹.h⁻¹).

Denitrification. Under anoxic conditions, a parameter η is used to lower the process rate either because denitrification occurs at a lower rate or because only a fraction η of PAO is capable of denitrification. In concept 1, the same amount of PHA is used under aerobic or anoxic conditions, whereas in concept 2 more energy is required under anoxic conditions to store the same amount of polyP, because the energy production efficiency is lower with nitrate than with oxygen (Mino *et al.*, 1998).

Glycogen storage. Glycogen storage is considered only in the ASM2d+TUD metabolic model and is modelled as a result of PHA oxidation in the same way as described above.

Kinetics. When PAOs reach their maximum polyP storage potential, the phosphorus uptake is stopped.

3.3.2.3. Model limitations

Energy source. The stoichiometry of polyP formation and PAO growth processes in ASM2d and ASM3+BioP models are described as independent. However, experimental results show that oxidation of stored organic compounds (i.e. PHA) provides the energy for both PAO growth and polyP storage (Wentzel *et al.*, 1989). Therefore, ASM2d+TUD links both yields to energy production, whereas Barker & Dold and UCTPHO+ model PAO growth and polyP storage as a single process. This will impact the identifiability of the model parameters, which will make calibration more difficult in ASM2d and ASM3+BioP.

Denitrification. ASM2d and ASM3+BioP consider a constant yield for aerobic and anoxic processes, which is in contradiction with Barker and Dold's (1996) observations. In the same way as for OHO anoxic growth (3.2.3.3), using a single yield for polyP formation and PHA consumption under aerobic and anoxic conditions will lead to an overestimation of polyP storage and underestimation in PAO denitrification.

Glycogen storage. The model limitations occurring when glycogen storage or GAOs are neglected are discussed in paragraph 3.3.1.3 since they relate to anaerobic substrate uptake differences.

Phosphate precipitation. Under certain conditions, such as high pH (>7.5) and high Ca^{2+} or metals concentration, chemical precipitation of phosphorus (e.g. calcium phosphate) cannot be neglected in comparison with the BioP removal process. Phosphate precipitation is favoured by high local phosphate concentrations in anaerobic tanks due to phosphate release by PAOs. Under these conditions, a biologically induced phosphorus precipitation process should be considered to correctly predict the phosphorus removal (Maurer and Gujer, 1998; Maurer and Boller, 1999). In case of chemical phosphorus removal (by adding e.g. iron, aluminium or calcium salts) a chemical precipitation model also needs to be added.

3.3.3. PAO growth

3.3.3.1. Knowledge

The carbon source and energy for PAO growth are provided by PHA oxidation (Comeau *et al.*, 1986). PAOs have to compete with GAOs for substrate uptake under anaerobic conditions in order to form the PHA that is oxidised under aerobic/anoxic conditions. To be competitive, the first priority of PAOs is to resupply their storage pools. However, this cyclic storage and consumption of storage pools leads to energy wastage. Consequently, PAOs have a growth yield that is 13% lower than that of OHOs growing on the same substrate (Mino *et al.*, 1998).

Substrate. When S_{VFA} are present under aerobic conditions, Comeau *et al.* (1987) and Wentzel *et al.* (1989) observed both a direct growth of PAO on S_{VFA} and storage of S_{VFA} linked to phosphate release.

Nutrient source. Wentzel *et al.* (1989) observed the ability of PAO organisms to use nitrate as nitrogen source in case of ammonia depletion, with no modification of their kinetic behaviour. In case PO_4 becomes limiting, Wentzel *et al.* (1989) observed that growth continued and hypothesised that PAO can use their cell internal polyP storage as phosphorus source.

Denitrification. Some PAOs are able to use nitrate as an electron acceptor to oxidise stored carbon (Wentzel *et al.*, 1989). Experiments using different methods (molecular tools, chemical analysis, etc) have been carried out to determine whether denitrifying PAOs are distinct from non-denitrifying PAOs, but no consensus has been reached so far (Oehmen *et al.*, 2007). Recent studies show that some sub-groups of PAOs are capable to use only nitrite and other sub-groups are capable to use both nitrate and nitrite (Oehmen *et al.*, 2010). Growth yields depend on the electron acceptor because energy production efficiency is lower with nitrate than with oxygen (Mino *et al.*, 1998).

Kinetics. Brdjanovic *et al.* (1998b) showed that PAO growth does not depend on the SRT, but on the PHA conversion rate and on the PHA storage capacity, provided that a sufficient minimum SRT is attained.

3.3.3.2. Modelling

Two main concepts are used in the seven published models (Table 14):

- In the first concept, PAO growth is similar to OHO growth and the process is separated from polyP storage (ASM2d, ASM3+BioP, ASM2d+TUD).
- In the second concept, followed by UCTPHO⁺ and Barker & Dold, phosphate uptake is simultaneous to growth: PAOs take up phosphate as nutrient for growth and store it as energy source. Barker & Dold consider two polyP storage pools (low and high molecular weight), whereas UCTPHO⁺ considers a single polyP storage pool.

845 Substrate. All models consider PHA as the only carbon source for PAO growth.

846 Nutrient source. In the UCTPHO+ and Barker & Dold models, nitrate can be used as nitrogen
 847 source in the case of ammonia depletion. In the case of phosphate depletion, PAOs will use
 848 their polyP storage as phosphorus source. In the Barker & Dold model, only the polyP storage
 849 compound with low molecular weight ($X_{PAO,PP,L0}$) can be used.

850 The Barker & Dold model does not consider potential NH_X or PO_4 depletion during anoxic
 851 PAO growth, because it was considered unlikely to have ammonia or phosphate depletion in
 852 an anoxic tank (Barker and Dold, 1997).

853 Denitrification. PAO denitrification is considered in all studied BioP models. As a
 854 simplification, all models consider a single homogenous population. A parameter η is used to
 855 lower the process rate either because denitrification occurs at a lower rate or because only a
 856 fraction η of PAO is capable of denitrification. This last concept is the one explicitly chosen
 857 in UCTPHO+. This way to model PAO denitrification has been successfully applied in
 858 several models, whereas the concept of two PAO populations leads systematically to the
 859 dominance of the aerobic PAOs (Hu *et al.*, 2007). Table 15 indicates whether the models use
 860 a different growth or polyP storage yield in aerobic and anoxic conditions.

861 Kinetics. All the models except ASM2d+TUD use the same kinetic growth concept as OHO,
 862 based on a maximum growth rate ($\mu_{PAO,Max}$). ASM2d+TUD bases the PAO growth on the
 863 consumption rate of PHA (q_{PHA_PAO}). This is consistent with the stoichiometric coefficients
 864 that are normalised to PHA, and the storage pool restoration concept (3.3.2.2).

3.3.3.3. Model limitations

The Barker & Dold model considers polyP with a high molecular weight. As already discussed in paragraph 3.3.1.3., this distinction may have been introduced to cope with glycogen depletion conditions that stopped substrate uptake.

Substrate. Should S_{VFA} be present under aerobic conditions, the studied models may lead to erroneous results. Indeed, the studied models consider that PAOs can only grow on organic stored compound whereas it seems that PAOs can grow directly on S_{VFA} substrate (Wentzel *et al.*, 1989). PAOs are then in competition with OHOs under aerobic and anoxic conditions for S_{VFA} uptake. This direct growth has been neglected because it was considered unlikely (and undesirable) that S_{VFA} remain available under aerobic conditions.

Nutrient source. For a WWTP with high nitrification efficiency and/or a high phosphorus removal, the aerobic tank may be limited in ammonia and/or phosphorus. However, PAOs seem able to use nitrate or nitrite and stored phosphorus as nutrients. Consequently, ASM2d, ASM3+BioP and ASM2d+TUD may lead to an underprediction of PAO growth under ammonia and/or phosphorus depletion.

Denitrification. Potential consequences in using single aerobic and anoxic yields are discussed in paragraph 3.3.2.3.

3.3.4. PAO decay

3.3.4.1. Knowledge

PAOs have the ability to store energy in the form of carbon (glycogen, PHA) or polyphosphates. These stored compounds make it essential to distinguish decay and maintenance in endogenous processes.

Decay is defined as the loss of biomass weight or activity due to internal cell factors or external factors, such as environmental conditions, viruses or predation (Lopez *et al.*, 2006; Hao *et al.*, 2010). Endogenous mass loss has been observed to be very low for PAOs compared to non-PAOs (Wentzel *et al.*, 1989). Also, Hao *et al.* (2009) found that the rate of cell death is far lower than the activity decay (i.e. reduction in specific activity rates). With all their storage polymers, PAOs “die” very slowly, and maintenance seems to be the main endogenous process.

Furthermore, experiments have shown that the PAOs decay rate is higher under aerobic conditions, and is low/negligible under anoxic and anaerobic conditions (Siegrist *et al.*, 1999; Lu *et al.*, 2007). The source of maintenance energy depends on the environmental conditions:

- Under aerobic conditions, PAOs use PHA, then glycogen (Brdjanovic *et al.*, 1998a; Lopez *et al.*, 2006; Lu *et al.*, 2007), but seem not able to use polyP for energy production (Lu *et al.*, 2007)
- Under anoxic conditions, PAOs use first PHA, which is rapidly depleted (Lopez *et al.*, 2006), then glycogen and polyP (Lu *et al.*, 2007). Experiments by Wentzel *et al.* (1989) showed the so-called secondary P-release during endogenous mass loss, due to polyP use.
- Under anaerobic conditions, PAOs would use both glycogen and polyP for maintenance (Lopez *et al.*, 2006; Lu *et al.*, 2007).

3.3.4.2. Modelling

Death-regeneration vs endogenous respiration. PAO decay is modelled according to the death-regeneration concept exclusively (ASM2d), as endogenous respiration exclusively (ASM3+BioP, ASM2d+TUD), or as a mix of the two concepts (Barker & Dold, UCTPHO+). In UCTPHO+, the death-regeneration concept is used under anoxic conditions only for PAOs

not able to use nitrate as electron acceptor (fraction $1-\eta$). Table 16 synthesises the concepts used in each model, depending on the electron acceptor conditions. The schematic representation of PAO decay and maintenance is shown in Table 17.

Electron acceptor conditions: In the Barker & Dold and UCTPHO+ models the maximum PAO decay rate is independent of the electron acceptor conditions, whereas two different decay rates are used under aerobic and anoxic conditions in the ASM2d+TUD model, and a reduction factor η_{mPAO} is used in ASM3+BioP.

Undegradable particulate matter production. Only ASM2d+TUD does not consider undegradable particulate matter production in the PAO decay process, because it is considered that insufficient experimental proof was available to evaluate this released material (Meijer, 2004).

Maintenance. This process is applied in the Barker & Dold, UCTPHO+ and ASM2d+TUD models. It consists exclusively in the cleavage of polyP to produce energy when oxygen is absent. The Barker & Dold and UCTPHO+ models also include polyP storage lysis, but it is not associated to energy production.

PAO storage pools lysis. The fate of PAO storage pools (PHA, glycogen, polyP) has to be modelled to ensure that the storage products decay together with the biomass (ASM2d, ASM3+BioP, Barker & Dold, UCTPHO+) (Table 18). In these lysis processes, storage compounds are usually released in the bulk phase into their initial form (VFAs for PHA and phosphate for polyP). However, UCTPHO+ considers that PHA is released as particulate biodegradable substrate. In ASM3+BioP, decay of the PHA storage pool is modelled as aerobic/anoxic PHA respiration and leads to total PHA oxidation.

Some models consider that the polyP storage pool lysis process does not produce energy, contrary to the maintenance process, and is considered to occur at the same rate as the biomass decay. The stoichiometry is however identical to the maintenance process. Table 19 synthesises the models that consider maintenance and/or polyP storage pool lysis.

ASM2d+TUD uses a maintenance concept and thus, the lysis of the storage pools do not appear directly, but are modelled with the aerobic and anoxic maintenance through PAO consumption.

3.3.4.3. Model limitations

Death-regeneration vs endogenous respiration. The limits highlighted for OHO and ANO decay processes (paragraph 3.2.5.3) also hold for the PAO decay process. In the death-regeneration concept, the released carbon (XC_B) from PAO biomass would first benefit OHOs (after hydrolysis). In the same way as the death-regeneration concept, PHA storage lysis of UCTPHO+ leads to XC_B release, which will benefit OHOs' growth first.

Electron acceptor conditions. The Barker & Dold and UCTPHO+ models consider the same decay rate under all electron acceptor conditions. However, the experimental results have shown that the anoxic and anaerobic decay may be neglected. Barker and Dold and UCTPHO+ models will thus lead to an overestimation of the PAO decay, and to an underestimation of the biological phosphorus removal. Suppressing the anoxic and anaerobic decay of PAO processes will solve the problem and simplify the model.

Maintenance. Only three models consider anaerobic maintenance (Barker & Dold, UCTPHO+ and ASM2d+TUD), whereas maintenance seems to be the main endogenous process for PAOs. Furthermore, only polyP is considered as a source of maintenance energy in these

models, while experiments also indicate the role of glycogen in the maintenance process (Lopez *et al.*, 2006; Lu *et al.*, 2007).

It should also be noted that aerobic maintenance is not considered explicitly. The maintenance energy needed is thus included in the aerobic growth process. This simplification could lead to an inadequate PAO biomass estimation in case of famine conditions (e.g. due to industrial activities interruption during the weekend).

4. DISCUSSION

4.1. Diversity of modelling concepts

ASM models have been proposed as mechanistic models that try to represent the biochemical transformations in activated sludge through several simplified process descriptions, as based on observed dynamics in WWTP. For the processes presented above no general consensus exists among modellers. Two main reasons can be mentioned:

- The main biochemical mechanisms included in the models are not yet fully understood and the models reflect the different hypotheses that were formulated.
- The mechanisms are too complex and models use different simplifications to reach the same agreement with measured data. However, this is at the expense of a clear mechanistic meaning of the models, and may limit the extrapolation potential of the models in some situations (e.g. industrial influents or extreme climates).

Table 20 synthesises all the modelling concepts used in the seven studied models, for each standard process. Only the ANO growth process is modelled identically in all considered models.

4.2. Theoretical limitations of models and choice of model

Table 21 synthetises the main theoretical limitations of models that have been highlighted in the "model limits" paragraph corresponding to each standard process. This table should help model users to choose a model adequate to the modelling objectives and to the environmental conditions of the WWTP to be modelled.

The modeller should first list the peculiarities of the WWTP's influent and of the treatment process (temperature, large anoxic or anaerobic zones...). This corresponds to the columns of Table 21. Second, the modeller should list the processes to be modelled with higher precision depending on the modelling objectives (corresponding to the rows of Table 21). Third, the modeller should check in the table whether some model limitations exist considering the environmental peculiarities and modelling objectives and list the adequate models for the modelling project. Fourth, for each limitation, the modeller should consult the corresponding paragraph in this paper for more explanations and potential model extensions that could overcome the standard model limitations.

Finally, the simpler model or the model that the modeller knows best in this list should be chosen.

4.3. Existing model modifications

Once the model is chosen, the user may have to include some modifications, either to reach the modelling objective (e.g. including multi-step nitrification and denitrification) or to cope with environmental conditions (e.g. modifying yields and kinetics depending on the electron acceptor), as underlined through this article for each process in the paragraphs "model limitations". When modifying an existing model, the user should be particularly careful on the following points:

- The stoichiometric continuity (Gujer and Larsen, 1995) and the kinetic consistency should be carefully checked using the method of Hauduc *et al.* (2010b), to ensure the mathematical accuracy of the model.

- As model processes often merge different mechanisms for simplification, the significance of other processes and parameters may change when adding (explicitly defined) or modifying some processes. For example, adding a storage process for OHO will lead to a different meaning of the hydrolysis process, and will lower the hydrolysis parameters (see discussion in paragraph 3.2.1.2). Consequently, model users should be very careful in using default model parameters in modified models.

4.4. Increased knowledge needed

This review of biochemical knowledge on biological processes and the comparison of the different modelling concepts highlighted some research needs. The knowledge gaps exist mainly in processes that have been simplified during the building of the ASM models (e.g. lumped processes, such as decay), because they were considered to be negligible or unlikely to occur in most situations. However, new wastewater treatment challenges have emerged and greater knowledge on some of these processes is required for a variety of applications. Consequently, these simplified descriptions of biological processes lead to conceptual uncertainties on the model structure that have been difficult to evaluate so far (Refsgaard *et al.*, 2007). The main issues to be addressed in future research are summarised in Table 22.

In addition, phosphorus precipitation (only considered in ASM2d but not be discussed in this article) would need further research to integrate the phenomenon of biologically induced phosphorus precipitation, which requires to model pH and other ions such as carbonate and magnesium (Maurer and Gujer, 1998; Maurer and Bollner, 1999; Barat *et al.*, 2011).

5. CONCLUSION

Activated sludge models have been published based on theoretical knowledge of process mechanisms. Seven of the most widely used models have been theoretically compared in

terms of their underlying modelling concepts. A schematic representation has been developed and applied to the modelling concepts for each standard process as an additional visualisation to complement the well-known Gujer Matrix notation.

First, this representation will help model users to better understand modelling concepts and model differences. This representation is complementary to the schematic model representation developed by Comeau and Takács (2008) that allows a global view of the model processes.

Secondly, this representation allows determining the main conceptual differences between models (modelling schools), and highlights their main theoretical limits that should be taken into account when selecting a model in a modelling project, among which:

- Component-based models (more flexible) versus fraction-based models (less complex),
- Constant yields or different yields (depending on the electron acceptor) impacting the biomass production and the electron acceptor consumption,
- Fermentation modelled as transformation or as anaerobic growth process impacting the biomass production in case of large anaerobic zones,
- Direct growth or growth on stored substrate will depend on the loading conditions (cyclic),
- Death-regeneration is simpler and adequate under anaerobic conditions, while endogenous respiration is closer to reality and applicable for secondary substrate use,
- Modelling glycogen adds model complexity but also completeness and
- Simultaneous PAO growth and polyP storage accurately represent the interactions between metabolic mechanisms.

Finally, this critical review allows highlighting the main research needs to increase the model quality. The main issues for carbon and nitrogen removal concern the role of predation in the

treatment process, especially in the hydrolysis and decay processes, the role and importance of substrate storage by OHO and the multiple-step nitrification-denitrification processes. Concerning PAO processes, the competition between PAO and GAO is not fully understood, as is the use of stored compounds for maintenance and the role of the TCA cycle in the anaerobic PAO metabolism.

6. ACKNOWLEDGEMENT

This work has been carried out in close collaboration with the IWA Good Modelling Practice Task Group. The GMP Task Group is sponsored by IWA and its home institutions (Black & Veatch, Cemagref, EnviroSim Associates Ltd., Kurita Water Industries Ltd., TU Vienna, Canada Research Chair in Water Quality modelling, Université Laval, Ecole Polytechnique of Montreal, EAWAG). The GMP task group would like to especially thank its sponsors Hydromantis Environmental Software Solutions, Inc. and MOSTforWATER N.V.

Hélène Hauduc is a Ph.D. student at Cemagref (France) and modelEAU (Université Laval, Québec, Canada).

Peter Vanrolleghem holds the Canada Research Chair in Water Quality Modelling.

7. REFERENCES

- Barat R., Montoya T., Seco A. and Ferrer J. (2011) Modelling biological and chemically induced precipitation of calcium phosphate in enhanced biological phosphorus removal systems. *Water Research*, **45**(12), 3744-3752.
- Barker P.S. and Dold P.L. (1995) COD and nitrogen mass balances in activated sludge systems. *Water Research*, **29**(2), 633-643.
- Barker P.S. and Dold P.L. (1996) Denitrification behaviour in biological excess phosphorus removal activated sludge systems. *Water Research*, **30**(4), 769-780.
- Barker P.S. and Dold P.L. (1997) General model for biological nutrient removal activated-sludge systems: Model presentation. *Water Environment Research*, **69**(5), 969-984.
- Beccari M., Dionisi D., Giuliani A., Majone M. and Ramadori R. (2002) Effect of different carbon sources on aerobic storage by activated sludge. *Water Science and Technology*, **45**(6), 157-168.
- Beun J.J., Dircks K., van Loosdrecht M.C.M. and Heijnen J.J. (2002) Poly-beta-hydroxybutyrate metabolism in dynamically fed mixed microbial cultures. *Water Research*, **36**(5), 1167-1180.
- Brdjanovic D., Slamet A., van Loosdrecht M.C.M., Hooijmans C.M., Alaerts G.J. and Heijnen J.J. (1998a) Impact of excessive aeration on biological phosphorus removal from wastewater. *Water Research*, **32**(1), 200-208.

- Brdjanovic D., van Loosdrecht M.C.M., Hooijmans C.M., Alaerts G.J. and Heijnen J.J. (1998b) Minimal aerobic sludge retention time in biological phosphorus removal systems. *Biotechnology and Bioengineering*, **60**(3), 326-332.
- Brdjanovic D., van Loosdrecht M.C.M., Hooijmans C.M., Mino T., Alaerts G.J. and Heijnen J.J. (1998c) Bioassay for glycogen determination in biological phosphorus removal systems. *Water Science and Technology*, **37**(4-5), 541-547.
- Buchan L. (1983) Possible biological mechanism of phosphorus removal. *Water Science and Technology*, **15**(3-4), 87-103.
- Comeau Y., Hall K.J., Hancock R.E.W. and Oldham W.K. (1986) Biochemical model for enhanced biological phosphorus removal. *Water Research*, **20**(12), 1511-1521.
- Comeau Y., Rabinowitz B., Hall K.J. and Oldham W.K. (1987) Phosphate release and uptake in enhanced biological phosphorus removal from waste-water. *Journal Water Pollution Control Federation*, **59**(7), 707-715.
- Comeau Y. and Takács I. (2008). Schematic representation of activated sludge models. In: *Proceedings of Water Environment Federation 81st Annual Technical Exhibition & Conference*, Chicago, IL, USA, October 18-22, 2008.
- Corominas L., Rieger L., Takács I., Ekama G., Hauduc H., Vanrolleghem P.A., Oehmen A., Gernaey K.V. and Comeau Y. (2010) New framework for standardized notation in wastewater treatment modelling. *Water Science and Technology*, **61**(4), 841-857.
- Curds C.R. (1971) Computer simulations of microbial population dynamics in the activated-sludge process. *Water Research*, **5**(11), 1049-1066.
- de Kreuk M.K., Kishida N., Tsuneda S. and van Loosdrecht M.C.M. (2010) Behavior of polymeric substrates in an aerobic granular sludge system. *Water Research*, **44**(20), 5929-5938.
- Dircks K., Beun J.J., Van Loosdrecht M., Heijnen J.J. and Henze M. (2001) Glycogen metabolism in aerobic mixed cultures. *Biotechnology and Bioengineering*, **73**(2), 85-94.
- Dold P.L., Ekama G.A. and Marais G.v.R. (1980) A general model for the activated sludge process. *Progress in Water Technology*, **12**(6), 47-77.
- Downing A.A., Painter H.A. and Knowles G. (1964) Nitrification in the activated-sludge process. *Journal of Institute of Sewage Purification*, **64**(2), 130-158.
- Ekama G.A. and Marais G.v.R. (1979) Dynamic behavior of the activated sludge process. *Journal of the Water Pollution Control Federation*, **51**(3 I), 534-556.
- Ekama G.A. and Wentzel M.C. (1999) Difficulties and developments in biological nutrient removal technology and modelling. *Water Science and Technology*, **39**(6), 1-11.
- Ekama G.A. and Wentzel M.C. (2004) Modelling inorganic material in activated sludge systems. *Water SA*, **30**(2), 153-174.
- Gernaey K.V., van Loosdrecht M.C.M., Henze M., Lind M. and Jørgensen S.B. (2004) Activated sludge wastewater treatment plant modelling and simulation: State of the art. *Environmental Modelling and Software*, **19**(9), 763-783.
- Goel R., Mino T., Satoh H. and Matsuo T. (1999) Modeling hydrolysis processes considering intracellular storage. *Water Science and Technology*, **39**(1), 97-105.
- Grady C.P.L., Gujer W. and Henze M. (1986) A model for single-sludge wastewater treatment systems. *Water Science and Technology*, **18**(6), 47-61.
- Guisasola A., Sin G., Baeza J.A., Carrera J. and Vanrolleghem P.A. (2005) Limitations of ASM1 and ASMS3: A comparison based on batch oxygen uptake rate profiles from different full-scale wastewater treatment plants. *Water Science and Technology*, **52**(10-11), 69-77.
- Gujer W. (2010) Nitrification and me - A subjective review. *Water Research*, **44**(1), 1-19.
- Gujer W., Henze M., Mino T. and van Loosdrecht M.C.M. (1999) Activated Sludge Model No. 3. *Water Science and Technology*, **39**(1), 183-193.
- Gujer W., Henze M., Mino T. and van Loosdrecht M.C.M. (2000). Activated Sludge Model No.3. In *Activated Sludge Models ASM1, ASM2, ASM2d and ASM3*. edited by M. Henze, et al., IWA Publishing, London, UK.
- Gujer W. and Larsen T.A. (1995) The implementation of biokinetics and conservation principles in ASIM. *Water Science and Technology*, **31**(2), 257-266.
- Hao X., Wang Q., Zhang X., Cao Y. and Mark Loosdrecht C.M.v. (2009) Experimental evaluation of decrease in bacterial activity due to cell death and activity decay in activated sludge. *Water Research*, **43**(14), 3604-3612.
- Hao X.D., Wang Q.L., Zhu J.Y. and van Loosdrecht M.C.M. (2010) Microbiological endogenous processes in biological wastewater treatment systems. *Critical Reviews in Environmental Science and Technology*, **40**(3), 239-265.
- Hauduc H., Gillot S., Rieger L., Ohtsuki T., Shaw A., Takács I. and Winkler S. (2009) Activated sludge modelling in practice - An international survey. *Water Science and Technology*, **60**(8), 1943-1951.

- 1137 Hauduc H., Rieger L., Ohtsuki T., Shaw A., Takács I., Winkler S., Heduit A., Vanrolleghem P.A. and Gillot S.
 1138 (2010a). Activated sludge modelling: Development and potential use of a practical applications database. In:
 1139 *Proceedings of 2nd IWA/WEF Wastewater Treatment Modelling Seminar*, Mont Sainte Anne, QC, Canada,
 1140 March 28-30 2010.
- 1141 Hauduc H., Rieger L., Takács I., Heduit A., Vanrolleghem P.A. and Gillot S. (2010b) A systematic approach for
 1142 model verification: Application on seven published activated sludge models. *Water Science and Technology*,
 1143 **61**(4), 825-839.
- 1144 Henze M., Grady C.P.L., Gujer W., Marais G.v.R. and Matsuo T. (1987) *Activated Sludge Model No. 1*,
 1145 IAWPRC Publishing, London, UK.
- 1146 Henze M., Grady C.P.L., Gujer W., Marais G.v.R. and Matsuo T. (2000a). Activated Sludge Model No.1. In
 1147 *Activated Sludge Models ASM1, ASM2, ASM2d and ASM3*. edited by M. Henze, et al., IWA Publishing, London,
 1148 UK.
- 1149 Henze M., Gujer W., Mino T., Matsuo T., Wentzel M.C., Marais G.v.R. and van Loosdrecht M.C.M. (1999)
 1150 Activated Sludge Model No.2d, ASM2d. *Water Science and Technology*, **39**(1), 165-182.
- 1151 Henze M., Gujer W., Mino T., Matsuo T., Wentzel M.C., Marais G.v.R. and van Loosdrecht M.C.M. (2000b).
 1152 Activated Sludge Model No.2d. In *Activated Sludge Models ASM1, ASM2, ASM2d and ASM3*. edited by M.
 1153 Henze, et al., IWA Publishing, London, UK.
- 1154 Herbert D., Elsworth R. and Telling R.C. (1956) The continuous culture of bacteria; a theoretical and
 1155 experimental study. *J. gen. Microbiol.*, **14**, 601-622.
- 1156 Hiatt W.C. and Grady C.P.L. (2008) An updated process model for carbon oxidation, nitrification, and
 1157 denitrification. *Water Environment Research*, **80**(11), 2145-2156.
- 1158 Hu Z.R., Wentzel M.C. and Ekama G.A. (2007) A general kinetic model for biological nutrient removal
 1159 activated sludge systems: Model development. *Biotechnology and Bioengineering*, **98**(6), 1242-1258.
- 1160 Jenkins D. and Tandoi V. (1991) The applied microbiology of enhanced biological phosphate removal
 1161 accomplishments and needs. *Water Research*, **25**(12), 1471-1478.
- 1162 Kaelin D., Manser R., Rieger L., Eugster J., Rottermann K. and Siegrist H. (2009) Extension of ASM3 for two-
 1163 step nitrification and denitrification and its calibration and validation with batch tests and pilot scale data. *Water*
 1164 *Research*, **43**(6), 1680-1692.
- 1165 Karahan-Gul O., van Loosdrecht M.C.M. and Orhon D. (2003) Modification of activated sludge model no. 3
 1166 considering direct growth on primary substrate. *Water Science and Technology*, **47**(11), 219-225.
- 1167 Kong Z., Vanrolleghem P.A., Willems P. and Verstraete W. (1996) determination of inhibition kinetics of
 1168 carbon oxidation and nitrification with a respirometer. *Water Research*, **30**, 825-836.
- 1169 Kraemer J., Constantine T., Crawford G., Erdal Z., Katehis D., Johnson B. and Daigger G. (2008). Process
 1170 design implications due to differences in anaerobic COD stabilisation models. In: *Proceedings of 81st Annual*
 1171 *Water Environment Federation Technical Exhibition and Conference*, Chicago, Illinois, USA, October 18-22,
 1172 2008.
- 1173 Krishna C. and van Loosdrecht M.C.M. (1999) Substrate flux into storage and growth in relation to activated
 1174 sludge modeling. *Water Research*, **33**(14), 3149-3161.
- 1175 Larrea L., Irizar I. and Hildago M.E. (2002) Improving the predictions of ASM2d through modelling in practice.
 1176 *Water Science and Technology*, **45**(6), 199-208.
- 1177 Lijklema L. (1973) Model for nitrification in activated sludge process. *Environmental Science and Technology*,
 1178 **7**(5), 428-433.
- 1179 Lopez-Vazquez C.M., Oehmen A., Hooijmans C.M., Brdjanovic D., Gijzen H.J., Yuan Z. and van Loosdrecht
 1180 M.C.M. (2009) Modeling the PAO-GAO competition: Effects of carbon source, pH and temperature. *Water*
 1181 *Research*, **43**(2), 450-462.
- 1182 Lopez C., Pons M.N. and Morgenroth E. (2006) Endogenous processes during long-term starvation in activated
 1183 sludge performing enhanced biological phosphorus removal. *Water Research*, **40**(8), 1519-1530.
- 1184 Lu H., Keller J. and Yuan Z. (2007) Endogenous metabolism of *Candidatus Accumulibacter phosphatis* under
 1185 various starvation conditions. *Water Research*, **41**(20), 4646-4656.
- 1186 Maurer M. and Boller M. (1999) Modelling of phosphorus precipitation in wastewater treatment plants with
 1187 enhanced biological phosphorus removal. *Water Science and Technology*, **39**(1), 147-163.
- 1188 Maurer M. and Gujer W. (1998) Dynamic modelling of enhanced biological phosphorus and nitrogen removal in
 1189 activated sludge systems. *Water Science and Technology*, **38**(1), 203-210.
- 1190 Meijer S.C.F. (2004) *Theoretical and practical aspects of modelling activated sludge processes*. PhD thesis,
 1191 Department of Biotechnological Engineering, Delft University of Technology, Delft, The Netherlands.
- 1192 Mino T., Kawakami T. and Matsuo T. (1985) Location of phosphorus in activated sludge and function of
 1193 intracellular polyphosphates in biological phosphorus removal process. *Water Science and Technology*, **17**(2-3 -
 1194 3 pt 1), 93-106.
- 1195 Mino T., van Loosdrecht M.C.M. and Heijnen J.J. (1998) Microbiology and biochemistry of the enhanced
 1196 biological phosphate removal process. *Water Research*, **32**(11), 3193-3207.

- Morgenroth E., Kommedal R. and Harremoes P. (2002) Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment - A review. *Water Science and Technology*, **45**(6), 25-40.
- Moussa M.S., Hooijmans C.M., Lubberding H.J., Gijzen H.J. and van Loosdrecht M.C.M. (2005) Modelling nitrification, heterotrophic growth and predation in activated sludge. *Water Research*, **39**(20), 5080-5098.
- Muller A., Wentzel M.C., Loewenthal R.E. and Ekama G.A. (2003) Heterotroph anoxic yield in anoxic aerobic activated sludge systems treating municipal wastewater. *Water Research*, **37**(10), 2435-2441.
- Munz G., Lubello C. and Oleszkiewicz J.A. (2011) Modeling the decay of ammonium oxidizing bacteria. *Water Research*, **45**, 557-564.
- Ni B.J., Rittmann B.E. and Yu H.Q. (2010) Modeling predation processes in activated sludge. *Biotechnology and Bioengineering*, **105**(6), 1021-1030.
- Nowak O., Svardal K., Franz A. and Kuhn V. (1999) Degradation of particulate organic matter - A comparison of different model concepts. *Water Science and Technology*, **39**(1), 119-127.
- Nowak O., Svardal K. and Schweighofer P. (1995) The dynamic behaviour of nitrifying activated sludge systems influenced by inhibiting wastewater compounds. *Water Science and Technology*, **31**(2), 115-124.
- Oehmen A., Lemos P.C., Carvalho G., Yuan Z., Keller J., Blackall L.L. and Reis M.A.M. (2007) Advances in enhanced biological phosphorus removal: From micro to macro scale. *Water Research*, **41**(11), 2271-2300.
- Oehmen A., Lopez-Vazquez C.M., Carvalho G., Reis M.A.M. and van Loosdrecht M.C.M. (2010) Modelling the population dynamics and metabolic diversity of organisms relevant in anaerobic/anoxic/aerobic enhanced biological phosphorus removal processes. *Water Research*, **44**(15), 4473-4486.
- Orhon D., Cokgor E.U. and Sozen S. (1998) Dual hydrolysis model of the slowly biodegradable substrate in activated sludge systems. *Biotechnology Techniques*, **12**(10), 737-741.
- Orhon D., Sozen S. and Artan N. (1996) The effect of heterotrophic yield on the assessment of the correction factor for anoxic growth. *Water Science and Technology*, **34**(5-6), 67-74.
- Pirt S.J. (1965) The maintenance energy of bacteria in growing cultures. *Proceedings of the Royal Society of London. Series B, Containing papers of a Biological character*. Royal Society (Great Britain), **163**(991), 224-231.
- Refsgaard J.C., van der Sluijs J.P., Højberg A.L. and Vanrolleghem P.A. (2007) Uncertainty in the environmental modelling process - A framework and guidance. *Environmental Modelling and Software*, **22**(11), 1543-1556.
- Rieger L., Koch G., Kuhni M., Gujer W. and Siegrist H. (2001) The EAWAG Bio-P module for Activated Sludge Model No. 3. *Water Research*, **35**(16), 3887-3903.
- Siegrist H., Brunner I., Koch G., Phan L.C. and Van Le C. (1999) Reduction of biomass decay rate under anoxic and anaerobic conditions. *Water Science and Technology*, **39**(1), 129-137.
- Sin G., Guisasola A., De Pauw D.J.W., Baeza J.A., Carrera J. and Vanrolleghem P.A. (2005) A new approach for modelling simultaneous storage and growth processes for activated sludge systems under aerobic conditions. *Biotechnology and Bioengineering*, **92**(5), 600-613.
- Sin G., Kaelin D., Kampschreur M.J., Takács I., Wett B., Gernaey K.V., Rieger L., Siegrist H. and van Loosdrecht M.C.M. (2008) Modelling nitrite in wastewater treatment systems: A discussion of different modelling concepts. *Water Science and Technology*, **58**(6), 1155-1171.
- Smolders G.J.F., van Der Meij J., van Loosdrecht M.C.M. and Heijnen J.J. (1994a) Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process. *Biotechnology and Bioengineering*, **44**(7), 837-848.
- Smolders G.J.F., van der Meij J., van Loosdrecht M.C.M. and Heijnen J.J. (1994b) Model of the anaerobic metabolism of the biological phosphorus removal process: Stoichiometry and pH influence. *Biotechnology and Bioengineering*, **43**(6), 461-470.
- Sozen S., Cokgor E.U., Orhon D. and Henze M. (1998) Respirometric analysis of activated sludge behaviour--II. Heterotrophic growth under aerobic and anoxic conditions. *Water Research*, **32**(2), 476-488.
- Sudiana I.M., Mino T., Satoh H., Nakamura K. and Matsuo T. (1999) Metabolism of enhanced biological phosphorus removal and non-enhanced biological phosphorus removal sludge with acetate and glucose as carbon source. *Water Science and Technology*, **39**(6), 29-35.
- Takács I. (2005) The Petersen/Petersen a.k.a. Gujer matrix – revisiting the Activated Sludge Model. *Water* **21**, February 2005.
- van Aalst-Van Leeuwen M.A., Pot M.A., van Loosdrecht M.C.M. and Heijnen J.J. (1997) Kinetic modeling of poly(β -hydroxybutyrate) production and consumption by *Paracoccus pantotrophus* under dynamic substrate supply. *Biotechnology and Bioengineering*, **55**(5), 773-782.
- van Loosdrecht M.C.M. and Heijnen J.J. (2002) Modelling of activated sludge processes with structured biomass. *Water Science and Technology*, **45**(6), 13-23.
- van Loosdrecht M.C.M. and Henze M. (1999) Maintenance, endogenous respiration, lysis, decay and predation. *Water Science and Technology*, **39**(1), 107-117.

- van Loosdrecht M.C.M., Hooijmans C.M., Brdjanovic D. and Heijnen J.J. (1997a) Biological phosphate removal processes. *Applied Microbiology and Biotechnology*, **48**(3), 289-296.
- van Loosdrecht M.C.M., Pot M.A. and Heijnen J.J. (1997b) Importance of bacterial storage polymers in bioprocesses. *Water Science and Technology*, **35**(1), 41-47.
- Vanrolleghem P.A., Kong Z. and Coen F. (1996) Full-scale on-line assessment of toxic wastewaters causing change in biodegradation model structure and parameters. *Water Science and Technology*, **33**(2), 163-175.
- Warner A.P.C., Ekama G.A. and Marais G.v.R. (1986) The activated sludge process. IV. Application of the general kinetic model to anoxic-aerobic digestion of waste activated sludge. *Water Research*, **20**(8), 943-958.
- Wentzel M.C., Ekama G.A., Loewenthal R.E., Dold P.L. and Marais G.v.R. (1989) Enhanced polyphosphate organism cultures in activated sludge systems. Part II: Experimental behaviour. *Water S.A.*, **15**(2), 71-88.
- Wentzel M.C., Ekama G.A. and Marais G.v.R. (1992) Processes and modelling of nitrification denitrification biological excess phosphorus removal systems - A review. *Water Science and Technology*, **25**(6), 59-82.
- Wentzel M.C., Lotter L.H., Loewenthal R.E. and Marais G.v.R. (1986) Metabolic behaviour of *Acinetobacter* spp. in enhanced biological phosphorus removal - a biochemical model. *Water SA*, **12**(4), 209-224.
- Zhou Y., Pijuan M., Oehmen A. and Yuan Z. (2010) The source of reducing power in the anaerobic metabolism of polyphosphate accumulating organisms (PAOs) – a mini-review *Water Science and Technology*, **61**(7), 1653–1662.

8. CAPTIONS

- Table 1. List of studied activated sludge models
- Table 2. List of processes and models considered
- Table 3. Symbols used for kinetic expressions: examples
- Table 4. State variables used in the models and their composition in terms of ThOD (C), nitrogen (N) and phosphorus (P), indicated by their background introduced in Figure 1.
- Table 5. Hydrolysis of particulate substrate
- Table 6. Fermentation process
- Table 7. OHO growth process concepts
- Table 8. Stoichiometry for OHO growth
- Table 9. ANO growth process
- Table 10. OHO and ANO decay process concepts
- Table 11. Simplified representation of phosphorus accumulating organisms growth
- Table 12. PHA storage process concept
- Table 13. PolyP storage process concept
- Table 14. PAO growth
- Table 15. Synthesis of anoxic and aerobic yields used by each model

1292	Table 16. Synthesis of decay concept used in each model, depending on the electron acceptor
1293	condition
1294	Table 17: PAO decay process concepts
1295	Table 18: PAO storage pools release/consumption during lysis
1296	Table 19. Synthesis of polyP storage pool fate associated with PAO decay
1297	Table 20. Synthesis of modelling concepts for each of the standard processes
1298	Table 21. Synthesis of theoretical limitations of models for each standard process
1299	Table 22. Knowledge gaps and needs in research for practical advances and better
1300	understanding of activated sludge processes
1301	
1302	Figure 1. Symbols used for the schematic representation
1303	Figure 2. OHOs use of organic substrate as carbon and energy source
1304	Figure 3. Simplified metabolism of autotrophic bacteria
1305	Figure 4. PHA storage: biochemical details (adapted from Wentzel et al., 2008).
1306	

Table 1. List of studied activated sludge models

Models	Reference	Substrates	# of processes	# of state variables	# of interacting processes vs. variables	# of parameters												
						Total	Composition matrix parameters	Temperature adjustment	stoichiometry					kinetic				
									Hydrolysis	OHO	ANO	PAO	Biomass general	Hydrolysis	OHO	ANO	PAO	Biomass general
ASM1	Henze <i>et al.</i> , 2000a ⁽¹⁾	CN	8	13	31	26	2	7	-	1	1	-	1	3	6	5	-	-
Barker&Dold	Barker and Dold, 1997	CNP	36	19	153	81	16	18	2	5	2	8	-	4	9	5	11	1
ASM2d	Henze <i>et al.</i> , 2000b ⁽²⁾	CNP	21	19	136	74	13	12	1	1	1	3	1	6	12	6	18	-
ASM3	Gujer <i>et al.</i> , 2000 ⁽³⁾	CN	12	13	72	46	8	10	1	4	1	-	1	2	13	6	-	-
ASM3+BioP	Rieger <i>et al.</i> , 2001	CNP	23	17	148	83	15	13	1	4	1	5	1	2	13	7	21	-
UCTPHO+	Hu <i>et al.</i> , 2007	CNP	35	16	169	66	12	10	-	3	2	7	-	-	13	4	14	1
ASM2d+TUD	Meijer, 2004	CNP	22	18	154	98	16	15	1	2	2	12	-	6	12	6	26	-

N.B.: Chemical P precipitation not considered. Biomass general refers to common parameters to OHOs, ANOs and PAOs. ⁽¹⁾ first published in Henze *et al.*, 1987; ⁽²⁾ first published in Henze *et al.*, 1999; ⁽³⁾ first published in Gujer *et al.*, 1999.

Table 2. List of processes and models considered

Processes types	ASM1	Barker & Dold	ASM2d	ASM3	ASM3+ BioP	UCTPHO+	ASM2d +TUD
Hydrolysis	X	X	X	X	X		X
Fermentation		X	X			X	X
OHO growth	X	X	X	X	X	X	X
Adsorption						X	
Storage				X	X		
ANO growth	X	X	X	X	X	X	X
OHO & ANO decay	X	X	X	X	X	X	X
PHA storage		X	X		X	X	X
Glycogen storage							X
PolyP storage		} X	X		X	} X	X
PAO growth			X		X		X
PAO decay		X	X		X	X	X

Table 3. Symbols used for kinetic expressions: examples

Description	Notation	Symbol
Kinetic coefficients: maximum specific growth rate	μ_{OHO}	μ_{OHO}
Concentration of S_{NOx}	S_{NOx}	S_{NOx}
Monod function with S_{B} as substrate	$\frac{S_{\text{B}}}{K_{\text{SB}} + S_{\text{B}}}$	$M(S_{\text{B}})$
Inhibition Monod function with S_{NOx} as electron acceptor	$\frac{K_{\text{NOx}}}{K_{\text{NOx}} + S_{\text{NOx}}}$	$\text{IM}(S_{\text{NOx}})$
Monod function with S_{PO4} as substrate, only used in models considering phosphorus removal	$\frac{S_{\text{PO4}}}{K_{\text{PO4}} + S_{\text{PO4}}}$	$\langle M(S_{\text{PO4}}) \rangle$
Electron acceptor conditions (ex: OHO growth) (aerobic or anoxic conditions)		$\left\langle \frac{M(S_{\text{O2}})}{\eta_{\mu_{\text{OHO}}, \text{Ax}}} M(S_{\text{NOx}}) \cdot \text{IM}(S_{\text{O2x}}) \right\rangle$

N.B.: The symbol $\langle \rangle$ is used to indicate optional or alternative terms, one or none of the lines apply for the given condition.

Table 4. State variables used in the models and their composition in terms of ThOD (C), nitrogen (N) and phosphorus (P), indicated by their background introduced in Figure 1.

Description	Notation	Unit	ASM1	ASM2d	ASM3	ASM3 + BioP	Barker & Dold	UCT PHO+	ASM2d+ TUD
			CNP	CNP	CNP	CNP	CNP	CNP	CNP
COD soluble									
Soluble biodegradable organics	S_B	g COD.m ⁻³	X		XX	XXX			
Fermentable organic matter	S_F	g COD.m ⁻³		XXX			X	XXX	XXX
Fermentation product (volatile fatty acids)	S_{VFA}	g COD.m ⁻³		X			X	X	X
Soluble undegradable organics	S_U	g COD.m ⁻³	X	XXX	XX	XXX	X	XXX	XXX
Dissolved oxygen	S_{O2}	- g COD.m ⁻³	X	X	X	X	X	X	X
COD particulate and colloidal									
Particulate and colloidal biodeg. organics	X_{CB}	g COD.m ⁻³	X	XXX	XX	XXX	X	XXX	XXX
Adsorbed slowly biodegradable substrate	X_{Ads}	g COD.m ⁻³						XXX	
Particulate undegradable organics	X_U	g COD.m ⁻³		XXX	XX	XXX			XXX
Particulate undeg. organics from influent	$X_{U,Inf}$	g COD.m ⁻³	X				X	XXX	
Particulate undeg. endogenous products	$X_{U,E}$	g COD.m ⁻³	XX				XXX	XXX	
Nitrogen (N) and Phosphorus (P)									
Ammonium and ammonia nitrogen	S_{NHx}	g N.m ⁻³	X	X	X	X	X	X	X
Nitrate and nitrite	S_{NOx}	g N.m ⁻³	XX	XX	XX	XX	XX	XX	XX
Dissolved nitrogen gas	S_{N2}	g N.m ⁻³		XX	XX	XX			XX
Particulate and colloidal biodeg. organic N	$X_{CB,N}$	g N.m ⁻³	X				X		
Soluble biodegradable organic N	$S_{B,N}$	g N.m ⁻³	X				X		
Soluble undegradable organic N	$S_{U,N}$	g N.m ⁻³					X		
Soluble inorganic phosphate	S_{PO4}	g P.m ⁻³		X		X	X	X	X
Biomass									
Ordinary heterotrophic organisms	X_{OHO}	g COD.m ⁻³	X	X	X	X	X	X	XX
Autotrophic nitrifying organisms	X_{ANO}	g COD.m ⁻³	X	X	X	X	X	X	XX
Phosphorus accumulating organisms	X_{PAO}	g COD.m ⁻³		X		X	X	X	XX
Internal cells products									
Storage compound in OHOs	$X_{OHO,Stor}$	g COD.m ⁻³			X	X			
Storage compound in PAOs	$X_{PAO,Stor}$	g COD.m ⁻³		X		X	X	X	
Stored poly-β-hydroxyalkanoates in PAOs	$X_{PAO,PHA}$	g COD.m ⁻³							X
Stored glycogen in PAOs	$X_{PAO,Gly}$	g COD.m ⁻³							X
Stored polyP in PAOs	$X_{PAO,PP}$	g P.m ⁻³		X		X		X	X
Releasable stored polyP	$X_{PAO,PP,Lo}$	g P.m ⁻³					X		
Non-releasable stored polyP	$X_{PAO,PP,Hi}$	g P.m ⁻³					X		
Other									
Alkalinity (CaCO ₃)	S_{Alk}	mol CaCO ₃ .m ⁻³	X	X	X	X			X
Total suspended solids	X_{TSS}	g TSS.m ⁻³		X	X	X			X

Table 5. Hydrolysis of particulate substrate

Concept 1a: One step hydrolysis with organic N and C considered separately		Concept 1b: One step hydrolysis with N and P linked to organic matter	
Ox Ax Ox Ax An	a. ASM1 b. Barker & Dold	Ox Ax An Ox Ax An Ox Ax An	c. ASM2d d. ASM3 e. ASM3 + BioP

Organics:

a: $q_{XC_B-SB,hyd} \cdot M\left(\frac{XC_B}{X_{OHO}}\right) \cdot \left\langle \frac{M(S_{O_2})}{\eta_{qhyd,Ax} \cdot M(S_{NOx}) \cdot IM(S_{O_2})} \right\rangle \cdot X_{OHO}$

b, c: $q_{XC_B-SB,hyd} \cdot M\left(\frac{XC_B}{X_{OHO}}\right) \cdot \left\langle \frac{M(S_{O_2})}{\eta_{qhyd,Ax} \cdot M(S_{NOx}) \cdot IM(S_{O_2})} \right\rangle \cdot X_{OHO}$

d, e: $q_{XC_B-SB,hyd} \cdot M\left(\frac{XC_B}{X_{OHO}}\right) \cdot X_{OHO}$

Particulate nitrogen hydrolysis:

a, b: $q_{XC_B-SB,hyd} \cdot M\left(\frac{XC_{B,N}}{X_{OHO}}\right) \cdot \left\langle \frac{M(S_{O_2})}{\eta_{qhyd,Ax} \cdot M(S_{NOx}) \cdot IM(S_{O_2})} \right\rangle \cdot X_{OHO}$

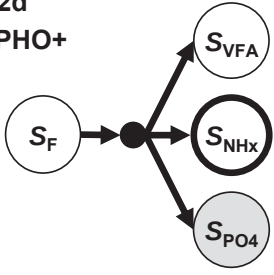
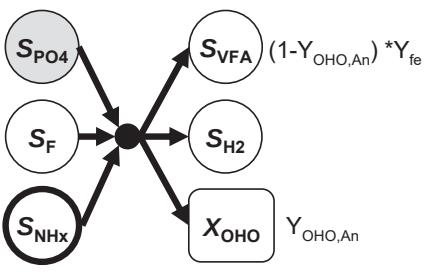
Ammonification:

a: $q_{am} \cdot S_{B,N} \cdot X_{OHO}$

b: $q_{am} \cdot S_{B,N} \cdot (X_{OHO} + X_{PAO})$

N.B.: in UCTPHO+, hydrolysis is considered simultaneously with growth. ASM2d+TUD is identical to ASM2d. The symbol $\langle \rangle$ is used to indicate optional or alternative terms, one or none of the lines should be chosen.

Table 6. Fermentation process

Concept 1: Transformation	Concept 2: Anaerobic growth process
An c. ASM2d An g. UCTPHO+  c: $q_{SF_VFA,Max} \cdot M(S_F) \cdot IM(S_{O_2}) \cdot IM(S_{NOx}) \cdot X_{OHO}$ g: $q_{SF_VFA,Max} \cdot S_F \cdot IM(S_{O_2}) \cdot IM(S_{NOx}) \cdot X_{OHO}$	An b. Barker & Dold  $q_{SF_VFA,Max} \cdot M(S_F) \cdot M(S_{NHx}) \cdot M(S_{PO4}) \cdot IM(S_{O_2}) \cdot IM(S_{NOx}) \cdot X_{OHO}$

N.B.: ASM2d+TUD is identical to ASM2d.

Table 7. OHO growth process concepts

Concept 1: direct growth		Concept 2: storage - growth
Concept 1a: S_{NHx} as only nitrogen source Adsorption Growth	Concept 1b: S_{NHx} or S_{NOx} as nitrogen source Adsorption Growth	Storage Growth
Concept 1a: S_{NHx} as only nitrogen source Adsorption Growth	Concept 1b: S_{NHx} or S_{NOx} as nitrogen source Adsorption Growth	Storage: Growth:

Table 8. Stoichiometry for OHO growth

Models	Organic substrate				Nitrogen source		Different anoxic growth yield
	$S_{VF\text{A}}$	S_F / S_B	X_{Ads}	X_{Stor}	S_{NHx}	S_{NOx}	
ASM1		X			X		
Barker&Dold	X	X			X	X	X
ASM2d	X	X			X		
ASM3				X	X		X
ASM3+BioP				X	X		X
UCTPHO+	X	X	X		X	X	X
ASM2d+TUD	X	X			X		X

Table 9. ANO growth process

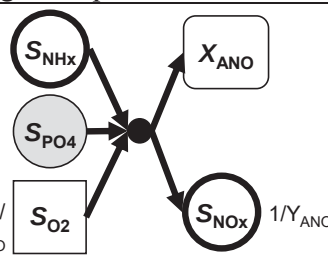
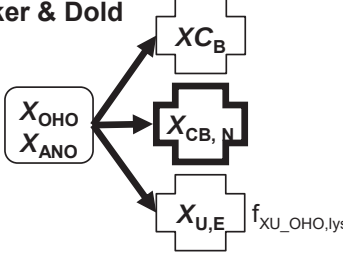
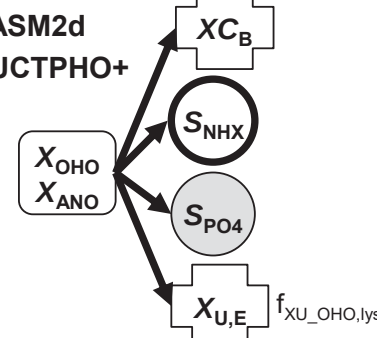
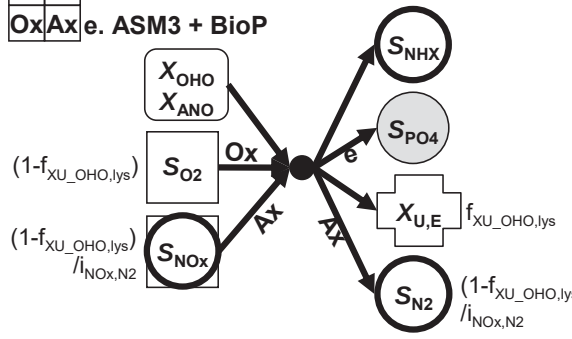
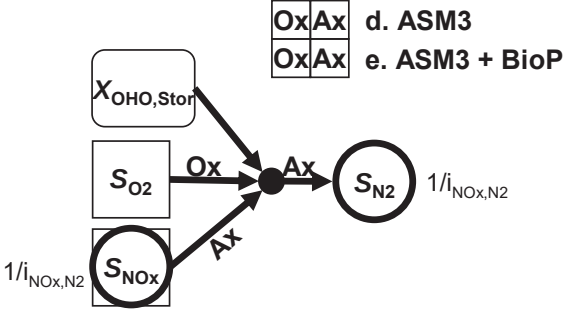
Ox	a. ASM1	
Ox	b. Barker & Dold	
Ox	c. ASM2d	
Ox	d. ASM3	
Ox	e. ASM3 + BioP	
Ox	f. ASM2d + TUD $(i_{\text{COD_NOx}} - Y_{\text{ANO}})/Y_{\text{ANO}}$	
Ox	g. UCTPHO+	
$\mu_{\text{ANO},\text{Max}} \cdot M(S_{\text{NHx}}) \cdot \langle M(S_{\text{PO4}}) \rangle \cdot M(S_{\text{O2}}) \cdot X_{\text{ANO}}$		

Table 10. OHO and ANO decay process concepts

Concept 1: Death-Regeneration concept	Concept 2: Endogenous respiration concept
Concept 1a: nutrients considered separately from substrate a. ASM1 b. Barker & Dold  Concept 1b: nutrients linked to substrate c. ASM2d g. UCTPHO+  Heterotrophs: $b_{OHO} \cdot X_{OHO}$ Autotrophs: $b_{ANO} \cdot X_{ANO}$	d. ASM3 e. ASM3 + BioP  Storage lysis (OHOs only) d. ASM3 e. ASM3 + BioP  Heterotrophs: $\left\langle \frac{m_{OHO,Ox} \cdot M(S_{O_2})}{m_{OHO,Ax} \cdot M(S_{NOx}) \cdot IM(S_{O_2})} \right\rangle \cdot X_{OHO}$ Storage lysis (OHOs only) $\left\langle \frac{m_{Stor,Ox} \cdot M(S_{O_2})}{m_{Stor,Ax} \cdot M(S_{NOx}) \cdot IM(S_{O_2})} \right\rangle \cdot X_{OHO,Stor}$ Autotrophs: $\left\langle \frac{m_{ANO,Ox} \cdot M(S_{O_2})}{m_{ANO,Ax} \cdot M(S_{NOx}) \cdot IM(S_{O_2})} \right\rangle \cdot X_{ANO}$

N.B.: ASM2d+TUD is identical to ASM2d.

The symbol $\langle \rangle$ is used to indicate optional or alternative terms, one or none of the lines should be chosen.

Table 11. Simplified representation of phosphorus accumulating organisms growth

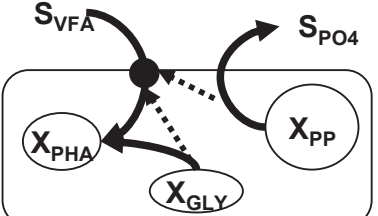
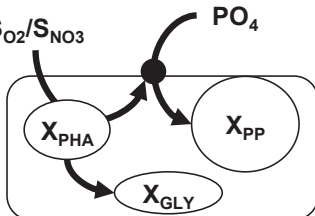
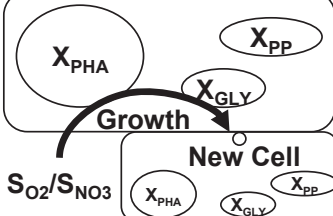
Anaerobic/Anoxic conditions	Aerobic/Anoxic conditions	
Substrate (S_{VFA}) uptake and storage in the form of PHA, with energy provided by glycogen and polyP breakdown, resulting in phosphate release	Storage pools restoration: phosphate uptake and glycogen formation	PAO growth, carbon and energy are provided by PHA storage pool oxidation
		

Table 12. PHA storage process concept

Concept 1: Energy from polyP, reducing power neglected	Concept 2: Energy from polyP, reducing power from glycogen
Ox Ax An Ox Ax An Ox Ax An Ox Ax An b. Barker & Dold c. ASM2d e. ASM3 + BioP g. UCTPHO+	Ax An f. ASM2d + TUD
b, g: $q_{PAO,VFA_Stor} \cdot M(S_{VFA}) \cdot M(X_{PAO,PP}) \cdot X_{PAO}$ c, e: $q_{PAO,VFA_Stor} \cdot \left\langle \frac{M(S_{VFA})}{M(S_B)} \right\rangle \cdot M(X_{PAO,PP} / X_{PAO}) \cdot X_{PAO}$	$\left\langle \frac{q_{PAO,VFA_PHA,An} \cdot IM(S_{O_2}) \cdot IM(S_{NOx}) \cdot M(X_{PAO,Gly})}{q_{PAO,VFA_PHA,Ax} \cdot IM(S_{O_2}) \cdot M(S_{NOx})} \right\rangle \cdot M(S_{VFA}) \cdot M(X_{PAO,PP}) \cdot X_{PAO}$

Table 13. PolyP storage process concept

Concept 1: Uncoupled processes	Concept 2: Coupled processes (metabolic model)
Ox Ax.c. ASM2d Ox Ax.e. ASM3 + BioP	PP storage: Ox Ax.f. ASM2d + TUD Glycogen storage: Ox Ax.f. ASM2d + TUD
$q_{P_{AO},PO_4,PP} \cdot \left(\eta_{q_{P_{AO},Ax}} \cdot IM(S_{O_2}) \cdot M(S_{NOx}) \right) \cdot M(S_{PO_4}) \cdot M(X_{P_{AO},PHA}) \cdot M(f_{PP-P_{AO},Max} - X_{P_{AO},PP} / X_{P_{AO}}) \cdot X_{P_{AO}}$	PP storage: $q_{P_{AO},PO_4,PP} \cdot \left(\eta_{q_{P_{AO},Ax}} \cdot IM(S_{O_2}) \cdot M(S_{NOx}) \right) \cdot [X_{P_{AO}} / X_{P_{AO},PP}]$ Glycogen storage: $q_{Gly} \cdot \left(\eta_{q_{P_{AO},Ax}} \cdot IM(S_{O_2}) \cdot M(S_{NOx}) \right) \cdot [X_{P_{AO},PHA} / X_{P_{AO},Gly}] \cdot M(X_{P_{AO},PHA}) \cdot M(f_{Gly-P_{AO},Max} - X_{P_{AO},Gly} / X_{P_{AO}}) \cdot X_{P_{AO}}$

NB: PolyP storage in the Barker & Dold and UCTPHO+ models are considered simultaneously with growth. The symbol < > is used to indicate optional or alternative terms, one or none of the lines should be chosen.

Table 14. PAO growth

Concept 1: PAO growth		
Ox Ax c. ASM2d Ox Ax e. ASM3 + BioP Ox Ax f. ASM2d + TUD	Concept 2a: Two polyP storage pools Ox Ax b. Barker & Dold	Concept 2b: Single polyP storage pools Ox Ax g. UCTPHO+
c. $1/Y_{Stor_PAO}$ $1-1/Y_{Stor_PAO}$ e. $1/Y_{Stor_PAO,OX}$ $1-1/Y_{Stor_PAO,OX}$ f. 1 $1-1/Y_{Stor_PAO,OX}$	A $1-1/Y_{Stor_PAO}$ B $1-1/Y_{Stor_PAO,OX}$ C 1	A $1-1/Y_{PAO,OX}$ B $1-1/Y_{PAO,OX}$ C 1
c, e: $\mu_{PAO_Max} \cdot \left(\eta_{PAO} \cdot IM(S_{O_2}) \cdot M(S_{NOx}) \right) \cdot M\left(\frac{X_{PAO,Stor}}{X_{PAO}} \right)$ d: $q_{PHI_PAO} \cdot \left(\eta_{PAO} \cdot IM(S_{O_2}) \cdot M(S_{NOx}) \right) \cdot M\left(\frac{X_{PAO,PHI}}{X_{PAO}} \right)$	A $Y_{Stor_PP,OX} \cdot Y_{PAO}$ B $Y_{Stor_PP,Ax} \cdot Y_{PAO}$ C 1	A $Y_{Stor_PP,OX} \cdot Y_{PAO}$ B $Y_{Stor_PP,Ax} \cdot Y_{PAO}$ C 1

Table[Click here to download Table: Table15.doc](#)

Table 15. Synthesis of anoxic and aerobic yields used by each model

Models	Aerobic / anoxic growth yield	Aerobic / anoxic polyP storage yield
Barker & Dold	Same	Different
ASM2d	Same	Same
ASM3+BioP	Different	Same
UCTPHO+	Different	Different
ASM2d+TUD	Different	Different

Table[Click here to download Table: Table16.doc](#)

Table 16. Synthesis of decay concept used in each model, depending on the electron acceptor condition

Models	Death-regeneration concept	Endogenous respiration concept
Barker & Dold		X
ASM2d	X	
ASM3+BioP		X
UCTPHO+	Anoxic (fraction $1-\eta$) Anaerobic	Aerobic Anoxic (fraction η)
ASM2d+TUD		X

Table 17: PAO decay process concepts

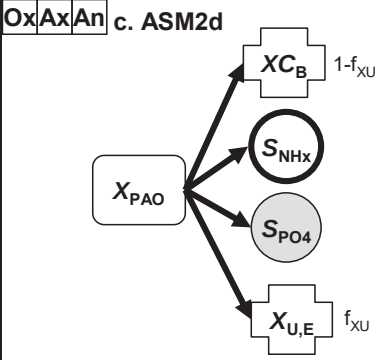
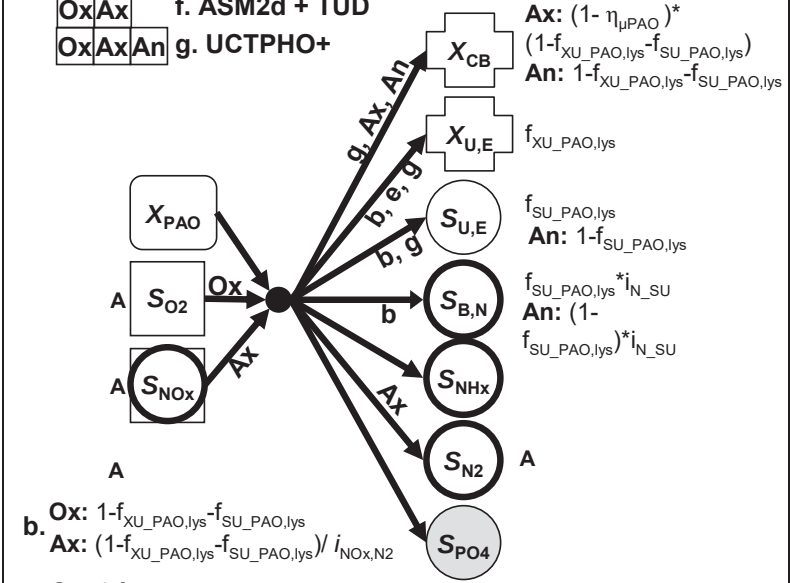
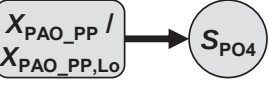
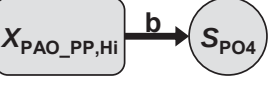
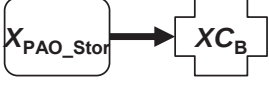
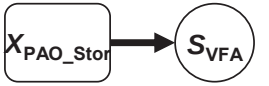
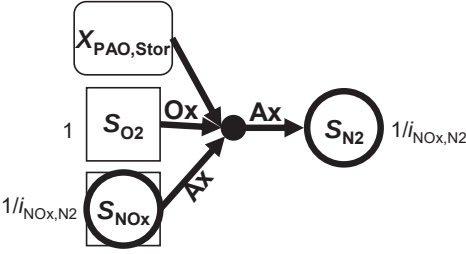
Concept 1: Death-regeneration concept	Concept 2: Endogenous respiration
c. ASM2d 	b. Barker & Dold e. ASM3 + BioP f. ASM2d + TUD g. UCTPHO+  Ax: $(1 - \eta_{\mu PAO})^*$ An: $(1 - f_{XU_PAO,lys} - f_{SU_PAO,lys})$ Ax: $(1 - f_{XU_PAO,lys} - f_{SU_PAO,lys})$ An: $1 - f_{XU_PAO,lys} - f_{SU_PAO,lys}$ Ax: $(1 - f_{XU_PAO,lys} - f_{SU_PAO,lys}) / i_{NOx,N2}$ An: $1 - f_{XU_PAO,lys} - f_{SU_PAO,lys}$ Ax: $1 - f_{XU_PAO,lys}$ An: $(1 - f_{XU_PAO,lys}) / i_{NOx,N2}$ Ax: 1 An: $1 / i_{NOx,N2}$ Ax: $1 - f_{XU_PAO,lys} - f_{SU_PAO,lys}$ An: $\eta_{\mu PAO} * (1 - f_{XU_PAO,lys} - f_{SU_PAO,lys}) / i_{NOx,N2}$ Maintenance: AxAn b. Barker & Dold AxAn f. ASM2d + TUD An g. UCTPHO+ $X_{PAO_PP} / X_{PAO_PP,Lo} \rightarrow S_{PO4}$
$b_{PAO} \cdot X_{PAO}$	Decay: b, g: $m_{PAO} \cdot \left\langle \frac{M(S_{O2})}{IM(S_{O2}) \cdot M(S_{NOx})} \right\rangle \cdot X_{PAO}$ e: $m_{PAO} \cdot \left\langle \frac{M(S_{O2})}{\eta_{mPAO} \cdot IM(S_{O2}) \cdot M(S_{NOx})} \right\rangle \cdot X_{PAO}$ f: $\left\langle \frac{m_{PAO,Ox} \cdot M(S_{O2})}{m_{PAO,Ax} \cdot IM(S_{O2}) \cdot M(S_{NOx})} \right\rangle \cdot X_{PAO}$ Maintenance: b: $b_{PP_PO4} \cdot IM(S_{O2}) \cdot M(X_{PAO,PP,Lo}) \cdot X_{PAO}$ f: $m_{PAO,An} \cdot IM(S_{O2}) \cdot IM(S_{NOx}) \cdot M(X_{PAO,PP}) \cdot X_{PAO}$ g: $b_{PP_PO4} \cdot \left\langle \frac{(1 - \eta_{\mu PAO}) \cdot IM(S_{O2}) \cdot M(S_{NOx})}{IM(S_{O2}) \cdot IM(S_{NOx})} \right\rangle \cdot M(X_{PAO,PP}) \cdot X_{PAO}$

Table 18: PAO storage pools release/consumption during lysis

Concept 1: Stored compounds are released	Concept 2: Stored compounds are consumed
PolyP lysis: Ox Ax An Ox Ax An Ox Ax An Ox Ax An b. Barker & Dold c. ASM2d e. ASM3 + BioP g. UCTPHO+   PHA lysis: Ox Ax An Ox Ax An Ox Ax An g. UCTPHO+ b. Barker & Dold c. ASM2d  	Ox Ax An e. ASM3 + BioP 
PolyP lysis: b, g: $m_{PAO} \cdot \left\langle \frac{M(S_{O_2})}{IM(S_{O_2}) \cdot IM(S_{NOx})} \right\rangle \cdot \left\langle \frac{X_{PAO,PP,Lo} / X_{PAO}}{X_{PAO,PP,Hi} / X_{PAO}} \right\rangle \cdot X_{PAO}$ c: $b_{PP_PAO} \cdot X_{PAO,PP}$ e: $b_{PP_PAO} \cdot \left\langle \frac{M(S_{O_2})}{\eta_{bPP_PAO} \cdot IM(S_{O_2}) \cdot M(S_{NOx})} \right\rangle \cdot X_{PAO,PP}$ PHA lysis: b: $m_{PAO} \cdot \left\langle \frac{M(S_{O_2})}{IM(S_{O_2}) \cdot M(S_{NOx})} \right\rangle \cdot \frac{X_{PAO,Stor}}{X_{PAO}} \cdot X_{PAO}$ c: $b_{Stor_PAO} \cdot X_{PAO,Stor}$ g: $m_{PAO} \cdot \left\langle \frac{M(S_{O_2})}{IM(S_{O_2}) \cdot M(S_{NOx})} \right\rangle \cdot M(S_{NH_4}) \cdot M(S_{PO_4}) \cdot \frac{X_{PAO,Stor}}{X_{PAO}} \cdot X_{PAO}$	$m_{PAO,Stor} \cdot \left\langle \frac{M(S_{O_2})}{\eta_{mPAO,Stor} \cdot IM(S_{O_2}) \cdot M(S_{NOx})} \right\rangle \cdot X_{PAO,Stor}$

Table[Click here to download Table: Table19.doc](#)

Table 19. Synthesis of polyP storage pool fate associated with PAO decay

Models	Maintenance by polyP cleavage	Lysis of polyP storage pool
Barker & Dold	X	X
ASM2d		X
ASM3+BioP		X
UCTPHO+	X	X
ASM2d+TUD	X	

Table 20. Synthesis of modelling concepts for each of the standard processes

	Concept 1	Concept 2
Hydrolysis	One step hydrolysis <i>Component-based model:</i> ASM1, Barker & Dold <i>Fraction-based model:</i> ASM2d, ASM3, ASM3+BioP	Direct growth on particulate substrate UCTPHO+
Fermentation	Transformation ASM2d, UCTPHO+, ASM2d+TUD	Anaerobic growth process Barker & Dold
OHO Growth	Direct growth on substrate <i>NH_x as only nitrogen source:</i> ASM1, ASM2d <i>NH_x/NO_x as nitrogen source:</i> Barker & Dold, UCTPHO+	Storage – Growth ASM3, ASM3+BioP
ANO Growth	One-step nitrification ASM1, Barker & Dold, UCTPHO+, ASM2d, ASM3, ASM3+BioP	
OHO & ANO decay	Death-regeneration <i>Component-based model:</i> ASM1, Barker & Dold <i>Fraction-based model:</i> ASM2d, UCTPHO+	Endogenous respiration ASM3, ASM3+BioP
PHA storage	Energy from polyP, reducing power neglected ASM2d, ASM3+BioP, UCTPHO+, Barker & Dold	Energy from polyP, reducing power from glycogen ASM2d+TUD
PolyP storage	Uncoupled processes ASM2d, ASM3+BioP	Coupled processes ASM2d+TUD
PAO growth	Growth ASM2d, ASM3+BioP, ASM2d+TUD	Simultaneous growth and polyP storage : Barker & Dold, UCTPHO+
PAO decay	Death-regeneration ASM2d	Endogenous respiration UCTPHO+, Barker & Dold, ASM2d+TUD, ASM3+BioP Maintenance: Barker & Dold, ASM2d+TUD, UCTPHO+
PAO storage pool lysis	PolyP lysis: Barker & Dold, ASM3+BioP, UCTPHO+ PHA lysis: UCTPHO+, Barker & Dold, ASM2d	Respiration of stored compounds ASM3+BioP

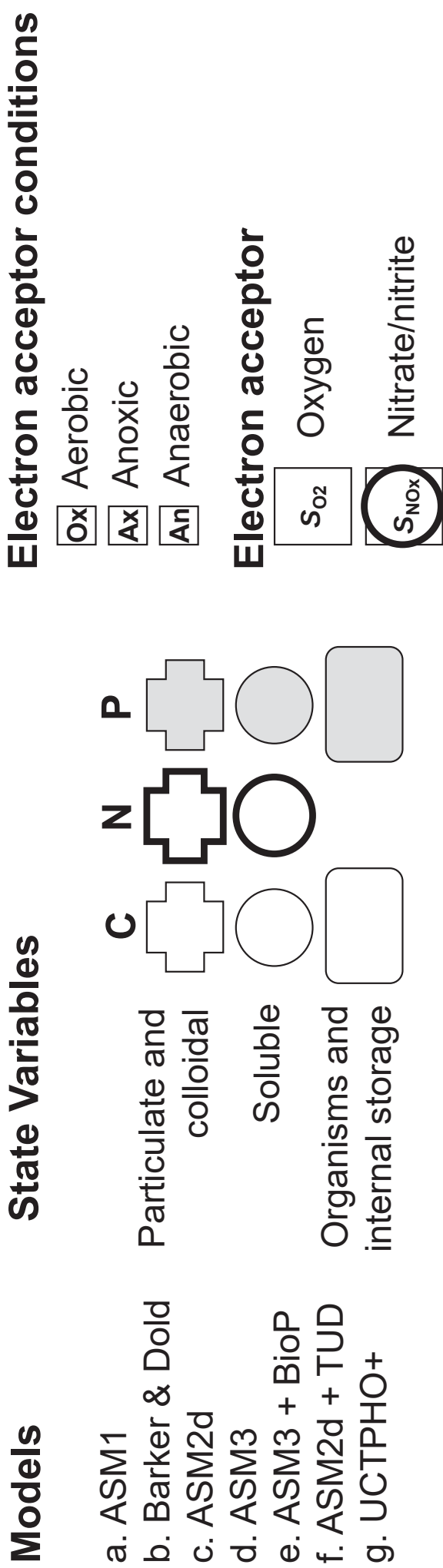
Table 21. Synthesis of theoretical limitations of models for each standard process

	State variables / Substrate	Nutrients	Aerobic conditions	Anoxic conditions	Anaerobic conditions	Simplified mechanisms
Hydrolysis (3.2.1.3)	X _{CB} as only particulate fraction	component-based models (ASM1 and Barker & Dold): ammonification			ASM1 and UCTPHO++ : anaerobic hydrolysis not considered	chemical dissolution, mass transport, storage (except ASM3 , ASM3+BioP)
Fermentation (3.2.2.3)					ASM2d and UCTPHO++ : anaerobic biomass production neglected	ASM3+BioP : fermentation neglected
OHO Growth (3.2.3.3)	See Table 8. - ASM3 : poor predictions for non constant growth and storage rates (SRT<5d, long feast/famine cycles...)	- ASM1 and ASM3 : P not limiting - UCTPHO++ , Barker & Dold : NO _x as N source	ASM3 : direct growth on S _B not considered	- ASM1 , ASM2d : constant yields - ASM3 : direct growth on S _B not considered		- one-step denitrification - inhibitory effects
ANO Growth (3.2.4.3)						one-step nitrification
OHO & ANO decay (3.2.5.3)	death-regeneration: substrate available only for OHOs				endogenous respiration concept (ASM3 , ASM3+BioP): anaerobic decay not considered	dormancy, maintenance, predation
PHA storage (3.3.1.3)	glycogen neglected (except ASM2d+TUD) ASM3+BioP : fermentation neglected					- competition with GAOs - effect of pH on acetate uptake (except ASM2d+TUD)
PolyP storage (3.3.2.3)	Barker & Dold : two polyP storage pools			ASM2d and ASM3+BioP : constant yields		biologically induced phosphate precipitation
PAO growth (3.3.3.3)	ASM2d , ASM3+BioP : growth and polyP storage not linked	UCTPHO++ and Barker & Dold only: NO _x as N source and polyP as P source	direct growth on S _{VFA} and S _F not considered	ASM2d and Barker & Dold : constant yield		
PAO decay (3.3.4.3)			aerobic maintenance not considered	Barker & Dold , UCTPHO++ : constant rate	- ASM3+BioP : anaerobic decay and anaerobic maintenance not modelled - Barker & Dold , UCTPHO++ : constant rate	PHA and glycogen utilisation for maintenance are not considered
PAO storage pool lysis (3.3.4.3)	UCTPHO++ : PHA released in the form of X _{CB}		ASM3+BioP : electron acceptor consumed	ASM3+BioP : electron acceptor consumed		ASM2d+TUD : Storage pool lysis not modelled

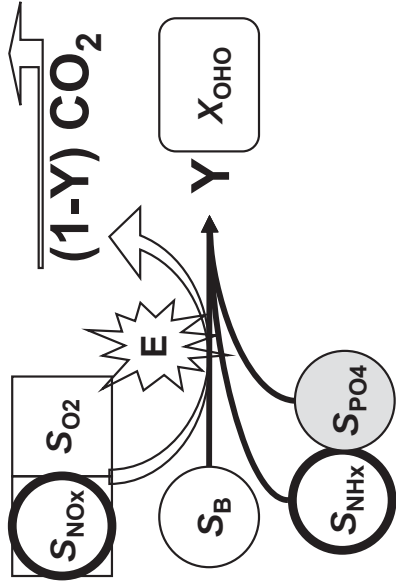
Table 22. Knowledge gaps and needs in research for practical advances and better understanding of activated sludge processes

Needs in research	Modelling process affected	Current modelling concept or solution	Modelling problem	Research needs in practice and in understanding
OHO and ANO processes				
Predation (e.g. protozoa activity, bacteriophage)	hydrolysis, biomass decay (concern PAO decay also)	not included in ASM type models. Hidden in calibrating hydrolysis parameters and decay rates.	varying hydrolysis and decay rates depending on the sludge age and other environmental conditions, especially the availability of electron acceptors	Needed for practical advances models have been developed but need to be validated. Experimental procedures to determine these model parameters and to quantify predation have to be developed.
Multiple-step denitrification	OHO growth under anoxic condition	one-step denitrification	current models cannot predict nitrite accumulation (shortcut nitrification-denitrification, inhibition problems...) and nitrous oxide (greenhouse gas issue)	Needed for practical advances some models exist, though further model development, integration with ASM models and validation are needed
Multiple-step nitrification	ANO growth	one-step nitrification		
OHO storage compounds and use	OHO growth and decay (maintenance)	storage considered only in ASM3 but simultaneous growth on external and stored substrate are not considered	poor predictions in case of low SRT (<5 d) and long feast/famine cycles	Needed for practical advances some models exist for both growth mechanisms, but need to be better integrated and validated
PAO processes				
GAO organisms	all PAO processes, P/VFA ratio in anaerobic period	not included in ASM type models. Hidden in calibrating non standard P/VFA ratio and other coefficients	effective BioP removal capacity of a plant	Needed for practical advances models exist and need to be integrated and validated at full-scale.
Maintenance mechanisms	PAO maintenance processes	polyP considered as only source of maintenance energy	the over/under prediction of phosphorus release, though the effect on modelling output is likely small	Needed for better understanding the preference of polyphosphate or glycogen for anaerobic maintenance processes should be elucidated.
TCA (tricarboxylic acid cycle) metabolism in PAO	P/VFA ratio in anaerobic period	calibrate the P/VFA ratio based on an accurate experiment	prediction of phosphorus release under anaerobic conditions, though likely also to be a small effect on model output	Needed for better understanding the relevance of the TCA cycle in anaerobic acetate uptake should be better understood.

Figure

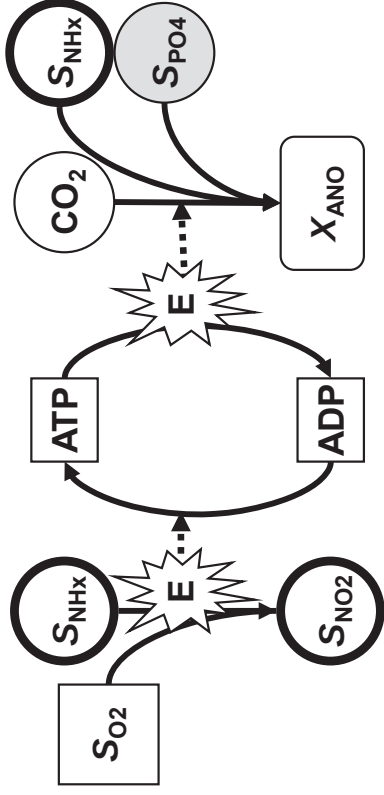


Figure

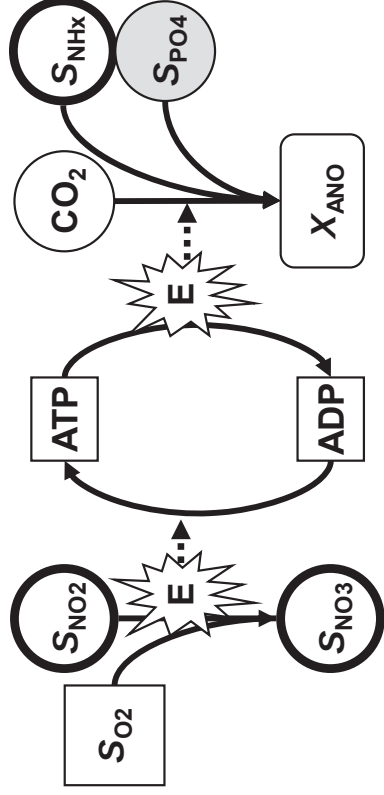


Figure

Nitrification



Nitrification



Figure

