REVIEW

BIOTECHNOLOGY and BIOENGINEERING

Critical Review of Activated Sludge Modeling: State of Process Knowledge, Modeling Concepts, and Limitations

H. Hauduc,[1,2] L. Rieger,[3] A. Oehmen,[4] M.C.M. van Loosdrecht,[5] Y. Comeau,[6] A. Héduit,[1] P.A. Vanrolleghem,[2] S. Gillot[1]

[1]Irstea, UR HBAN, 1 rue Pierre-Gilles de Gennes, F-92761 Antony Cedex, France; telephone: +33 (0)1 40 96 60 66; fax: +33 (0)1 40 96 61 99; e-mail: sylvie.gillot@irstea.fr

[2]modelEAU, Université Laval, Département de génie civil et de génie des eaux, Quebec (QC), Canada

[3]EnviroSim Associates Ltd., Hamilton (ON), Canada

[4]REQUIMTE/CQFB, Chemistry Department, Faculdade de Ciêcnias e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

[5]Department of Biotechnology, Delft University of Technology, Delft, The Netherlands

[6]Department of Civil, Geological and Mining Engineering, Polytechnique Montreal, Montreal, QC, Canada

ABSTRACT: This work critically reviews modeling 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 analyzed models are: (1) ASM1; (2) ASM2d; (3) ASM3; (4) ASM3 + BioP; (5) ASM2d + TUD; (6) Barker & Dold model; and (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. Standardized icons, based on shapes and color codes, enable the representation of the stoichiometric modeling 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.

Biotechnol. Bioeng. 2013;110: 24–46.

Additional Supporting Information may be found in the online version of this article.
Correspondence to: S. Gillot
Received 27 April 2012; Revision received 20 July 2012 Accepted manuscript online 7 August 2012;
Article first published online 5 September 2012 in Wiley Online Library (http://onlinelibrary.wiley.com/doi/10.1002/bit.24624/abstract)
DOI 10.1002/bit.24624

KEYWORDS: ASM; biological nutrient removal; modeling concepts; model selection

Introduction

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

Since the first publication of ASM1 (Grady et al., 1986), the biokinetic models are represented in a table format, which is named in practice Gujer matrix or Petersen matrix (Takács, 2005). This table contains a stoichiometric matrix and a kinetic vector. 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 (in columns), and all processes in which a state variable is involved (in rows). However, in case of large models such as ASM2d, it becomes difficult to "read" this matrix. Takács et al. (2011) proposed a schematic representation in which each model is represented in a single scheme (Comeau and Takács,

2008; Takács et al., 2011). 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, the comparison of models and particularly the concepts used is not convenient with this representation.

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).

In comparing these seven models, processes that only differ by the environmental conditions under which they take place were discussed together, resulting in identification of only nine major processes. The nine standard processes that will be discussed in separate sections of this article are the following: hydrolysis; fermentation; ordinary heterotrophic organisms (OHO) growth; autotrophic nitrifying organisms (ANO) growth; OHO & ANO decay; polyhydroxyalkanoates (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 modeling concepts. The major publications are cited for further reading. Then, (ii) the different modeling concepts used are compared through a new schematic representation of the stoichiometry and the kinetics (available as additional material). The standardised notation from Corominas et al. (2010) is used to help models comparison. 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 synthetizes the modeling concept diversity and the gray areas in theoretical knowledge, and discusses the model selection and existing model modifications.

Methodology

Studied Models

The seven published models have been chosen among those most commonly reported in literature (Table I). To keep the article 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

Table I. List of studied activated sludge models and their size in terms of number of processes, state variables and parameters.

						# of parameters											
									Stoichiometry				Kinetic				
Models	Refs.	Substrates	# of processes	# of state variables	# of interacting processes versus variables	Total	Composition matrix parameters	Temperature adjustment	Hydrolysis	OHO	ANO	PAO	Biomass general	Hydrolysis	OHO	ANO	PAO	Biomass general
ASM1	Henze et al. (2000a)[a]	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 et al. (2000b)[b]	CNP	21	19	136	74	13	12	1	1	1	3	1	6	12	6	18	—
ASM3	Gujer et al. (2000)[c]	CN	12	13	72	46	8	10	1	4	1	—	1	2	13	6	—	—
ASM3 + BioP	Rieger et al. (2001)	CNP	23	17	148	83	15	13	1	4	1	5	1	2	13	7	21	—
UCTPHO+	Hu et al. (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	—

Chemical P precipitation not considered. Biomass general refers to common parameters to OHOs, ANOs, and PAOs.
[a]First published in Henze et al. (1987).
[b]First published in Henze et al. (1999).
[c]First published in Gujer et al. (1999).

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., 2010).

Table I 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., 2011). Many parameters, particularly stoichiometric, seldom require adjustment. Some kinetic parameters (e.g., hydrolysis and decay processes) are routinely calibrated in practice. Understanding the different modeling approaches and their concepts greatly aids in determining when a parameter should be calibrated for a given situation.

Model Processes

Nine standard processes have been identified and are listed in Table II. 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 II synthesizes the standard processes included in each considered model and indicates the number of biological reactions merged in each standard process (e.g., hydrolysis process is described by five reactions in Barker & Dold model).

This work is limited to biological processes, and therefore chemical phosphorus precipitation is not discussed. Besides, 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.

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 modeling concept are represented on a single figure, and the standard processes that are different in terms of modeling concept are represented on separate figures. Different concepts are given different numbers (concept 1, concept 2...), whereas variations within the same concept are pointed out using letters (concept 1a, 1b...). The process is represented as a reaction with consumed components on the left of the figure and produced components on the right. The complete description and the schematic representation of the models for the nine standard processes are available as additional material.

Results

General Modeling Concepts

State Variables

All studied models are theoretical COD (ThOD) based. ThOD is the chosen organic material measure because it is a conservative quantity that allows characterizing 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.

Some conceptual differences among the studied models come from the state variables used. Table III summarizes for each model which state variables are considered and their composition in terms of ThOD (C), nitrogen (N), and phosphorus (P) (indicated by a cross under the respective columns for each model). Indeed, some models, named fraction-based models, consider that organic constituents represented by state variables expressed in ThOD contain also a nitrogen and a phosphorus fraction. These models are discussed in the following paragraph.

Table II. List of standard processes and number of biological reactions in each model.

Standard processes	ASM1	Barker & Dold	ASM2d[a]	ASM3	ASM3 + BioP	UCTPHO+	ASM2d + TUD
Hydrolysis	3	5	3	1	1		3
Fermentation		1	1			1	1
OHO growth	2	8	4	2	2	12	4
Adsorption						1	
Storage				2	2		
ANO growth	1	1	1	1	1	1	1
OHO and ANO decay	2	2	2	6	6	2	2
PHA storage		1	1		1	1	2
Glycogen storage							2
PolyP storage		} 5	2		2	} 6	2
PAO growth			2		2		2
PAO decay		13	3		6	11	3

[a]Without chemical precipitation processes.

Table III. State variables used in the models and their composition in terms of ThOD (C), nitrogen (N), and phosphorus (P), indicated by the "X" under their respective columns (e.g., S_U state variable is used in ASM3 and contains C and N fractions; S_{NO_x} is expressed in g N m^{-3} but contains negative ThOD).

			ASM1			ASM2d			ASM3			ASM3 + BioP			Barker & Dold			UCT PHO+			ASM2d + TUD		
Description	Notation	Unit	C	N	P	C	N	P	C	N	P	C	N	P	C	N	P	C	N	P	C	N	P
COD soluble																							
Soluble biodegradable organics	S_B	g COD m^{-3}	X						X	X		X	X	X									
Fermentable organic matter	S_F	g COD m^{-3}				X	X	X							X			X	X	X	X	X	X
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			X	X	X	X	X		X	X	X	X			X	X	X	X	X	X
Dissolved oxygen	S_{O_2}	$-$g COD m^{-3}	X			X			X			X			X			X			X		
COD particulate and colloidal																							
Particulate and colloidal biodeg. organics	XC_B	g COD m^{-3}	X			X	X	X	X	X		X	X	X	X			X	X	X	X	X	X
Adsorbed slowly biodegradable substrate	X_{Ads}	g COD m^{-3}																X	X	X			
Particulate undegradable organics	X_U	g COD m^{-3}				X	X	X	X	X		X	X	X							X	X	X
Particulate undeg. organics from influent	$X_{U,Inf}$	g COD m^{-3}	X												X			X	X	X			
Particulate undeg. endogenous products	$X_{U,E}$	g COD m^{-3}	X	X											X	X	X	X	X	X			
Nitrogen (N) and phosphorus (P)																							
Ammonium and ammonia nitrogen	S_{NH_x}	g N m^{-3}		X			X			X			X			X			X			X	
Nitrate and nitrite	S_{NO_x}	g N m^{-3}	X	X		X	X		X	X		X	X		X	X		X	X		X	X	
Dissolved nitrogen gas	S_{N_2}	g N m^{-3}				X	X		X	X		X	X								X	X	
Particulate and colloidal biodeg. org. N	$XC_{B,N}$	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_{PO_4}	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	X		X	X	X	X	X	X	X	X	X	X	X	X
Autotrophic nitrifying organisms	X_{ANO}	g COD m^{-3}	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Phosphorus accumulating organisms	X_{PAO}	g COD m^{-3}				X	X	X				X	X	X	X	X	X	X	X	X	X	X	X
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 PHA 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	

Component-Based/Fraction-Based Models

In the ASM1 and the Barker & Dold model, organic nitrogen is considered in separate state variables (component-based model). In these models nitrogen and phosphorus (Barker & Dold only) fractions are, however, linked to biomass. In other models, nitrogen, and phosphorus are linked to ThOD state variables (fraction-based models), as indicated

in Table III by crosses under respective columns N and P.

Component-based model. 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. However, in the Barker & Dold model, organic phosphorus is not considered as a state variable symmetrically to organic nitrogen. When released by biomass decay, phosphorus is then available in the form of o-PO_4 without any delay due to hydrolysis, contrary to organic nitrogen that has to be ammonified to be available in the form of NH_4^+.

Fraction-based model. Having linked variables limits the number of variables and processes. The substrate fraction is then supposed to be homogeneous and constant in its composition. However, in reality different organic compounds with different fractions are coming in the influent, which could induce a pitfall or increasing the model complexity by considering more components.

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.

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., 2010). This state variable needs to be added to check the continuity of the model and can be used to quantify denitrification.

Total Suspended Solids (TSS) as a State Variable

TSS is a combined variable in some models and a state variable in others (Table III), 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.

As total suspended solids (TSS) do not interact with processes and due to their simple calculation in the seven models considered, it has been chosen by the authors to not consider them in this study, in order to simplify the graphical representations.

Alkalinity

A low alkalinity value ($<50\,g\ CaCO_3\,m^{-3} = 1\,mol\ HCO_3^-\,m^{-3}$) results in an unstable pH (insufficient buffering capacity), which could cause nitrification inhibition and other process problems (Henze et al., 2000a). Alkalinity is thus modeled to predict the risk of pH limitation.

As alkalinity is not always considered in the models and is only indicating potential pH limitation, it has been chosen by the authors to not consider it in this study. For a deeper discussion on the use of alkalinity in ASM models, please refer to Hauduc et al. (2010).

In case pH modeling is of interest, different models based on chemical equilibrium of major ionic species have been developed (e.g., Serralta et al., 2004).

OHO and ANO Processes

Hydrolysis of Particulate Substrate

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 generalized.

Role of Protozoa. Protozoa seem able to take up particulate substrate and possibly release readily biodegradable substrate (de Kreuk et al., 2010). However, this process is so far poorly described.

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

Modeling. In the models studied here, only two concepts are used:

(1) One step hydrolysis concept, where slowly biodegradable substrate is hydrolyzed, then consumed by organisms. The differences between the models concern the way the residues of the reaction and the nitrogen fractions are modeled (see Component-Based/Fraction-Based Models Section):
    (1a) Component-based model (ASM1, Barker & Dold);
    (1b) Fraction-based model (ASM2d, ASM3, ASM3 + BioP).
(2) Direct growth concept (UCTPHO+) using adsorbed substrate. The hydrolysis is accounted for by a reduced growth rate for the use of this adsorbed substrate (see OHO growth process). This makes the hydrolyzed substrate available only for the organisms that produce hydrolytic enzymes, whereas in other modeling concepts the hydrolyzed substrate is released into the bulk phase, becoming available for all organisms, which will thus compete for it.

The hydrolysis process is 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 in Modeling under OHO and ANO Decay Section) merge those two types of hydrolysis in a single process, whereas in case of the endogenous respiration concept, the hydrolysis of secondary substrate is modeled through endogenous respiration and maintenance processes (ASM3 and ASM3 + BioP).

Electron acceptor conditions. The storage process and the utilization 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 the electron acceptor conditions in the hydrolysis kinetic rates. 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 in these models.

The Barker & Dold model introduces a hydrolysis yield under anoxic and anaerobic conditions to model the experimentally observed "COD loss" (Barker and Dold, 1995). Although this observation is not explained so far, the "loss" is modeled by H_2 gas formation (Kraemer et al., 2008). The S_{H_2} state variable is added to the model to reach continuity (Hauduc et al., 2010).

As UCTPHO+ models the hydrolysis process simultaneously with growth, anaerobic hydrolysis is not modeled.
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 modeled.
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 behavior 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 (Larrea et al., 2002; Nowak et al., 1999; Orhon et al., 1998).
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 (ASM1 and UCTPHO+). This is especially important for BioP models to make substrate available for PHA storage.
Experimental determination of parameters. Modeling 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).

Fermentation

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 oxidized to CO_2 and partially catabolized into volatile fatty acids (VFA, e.g., acetate), associated to organisms growth.
Modeling. Two different concepts are used to model fermentation in the studied models:

(1) The first concept considers fermentation as a transformation (ASM2d, UCTPHO+).
(2) 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 modeled by a S_{VFA} formation yield (Y_{fe}) in the fermentation process. The loss of $(1 - Y_{fe})$ g ThOD g S_{VFA}^{-1} is modeled 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., 2010).

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}). 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, for example, 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 growth during fermentation. Indeed, Ekama and Wentzel (1999) estimate the anaerobic growth yield at 0.10 g X_{OHO} g S_B^{-1}. In the case of large anaerobic zones, anaerobic growth may not be neglected and concept (2) should be chosen.

Ordinary Heterotrophic Organisms (OHO) Growth

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. This is schematically synthetized in Figure 1.

Substrate. van Loosdrecht et al. (1997b) proposed the existence of two types of bacteria. 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 cycles, 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.

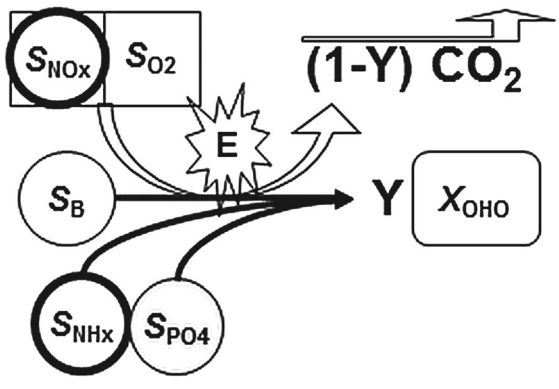


Figure 1. OHOs use organic substrate as carbon (with a yield *Y*) and energy source (*E*). Nitrate or oxygen is used as electron acceptor, and nutrients (ammonia, phosphates) are needed for growth.

Stored compounds, for example, 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).

The substrate uptake rate increases instantaneously when a high substrate concentration occurs (up to the maximum rate), but as the growth rate increases slowly, 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 (Beun et al., 2002; van Loosdrecht et al., 1997b).

Nutrients. Organism growth also requires nutrients such as nitrogen or phosphorus to synthetize 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 behavior. However, the yield will be slightly lower since some ThOD is used to reduce nitrate to ammonium.

Denitrification. Heterotrophic growth under anoxic conditions requires oxidized 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 ($Y_{OHO,Ax}$) to properly model the nitrate and COD consumptions was experimentally shown by several authors (Muller et al., 2003; Orhon et al., 1996; Sozen et al., 1998).

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

(1) Direct growth of OHOs on readily biodegradable substrate:
   (1a) NH_X is the only nitrogen source (ASM1, ASM2d),
   (1b) NO_X can be used as a nitrogen source in case of ammonia depletion (Barker & Dold, UCTPHO+).

UCTPHO+ considers adsorption of particulate substrate onto OHOs, followed by direct growth on the adsorbed substrate.

(2) The second concept considers substrate storage first 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 modeling. 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 and are summarized in Table IV:

- 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 hydrolyzed before use, which occurs simultaneously with growth. Modeling 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 hydrolyzed substrate (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., 2000a). The substrate preference switching function usually used in ASM models is in the form of the Equation (1) (with S_{Sub} being the considered substrate).

$$\left(\frac{S_{Sub}}{K_{S_{Sub}} + S_{Sub}}\right)\frac{S_{Sub}}{\sum_i S_{Sub,i}} \quad (1)$$

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

Denitrification. Denitrification is modeled as one step: nitrate is considered as the only possible electron acceptor. The maximum anoxic growth rate is lower than under aerobic conditions, either because $\mu_{OHO,Max}$ 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 (summarized in Table IV), since the efficiency of oxidative phosphorylation is lower under anoxic conditions.

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 days) (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 modeling 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 modeling 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 and ASM2d) could lead to an overestimation of the denitrification process in terms of biomass production, and underestimation of nitrogen removal and substrate consumption, which could 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

Table IV. Possible organic substrate and nitrogen sources for OHO growth, and stoichiometric anoxic growth yield.

	Organic substrate				Nitrogen source		Different anoxic growth yield
Models	S_{VFA}	S_F/S_B	X_{Ads}	X_{Stor}	S_{NH_x}	S_{NO_x}	
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

hydrolysis and storage processes to compensate the substrate consumption and maintain the experimentally observed denitrification rate (Muller et al., 2003).

Autotrophic Nitrifying Organisms (ANO) Growth

Knowledge. ANO oxidize ammonia to produce the required energy for CO_2 uptake and growth. This process is named nitrification. It includes two steps that involve two distinct groups of autotrophic organisms: ammonia oxidizers (ammonia to nitrite) and nitrite oxidizers (nitrite to nitrate). The simplified mechanism is schematically represented in Figure 2: ammonia and then nitrite are used as energy source (electron donors), which is transferred in the form of ATP in the cell. This energy is then used to reduce carbon dioxide into biomass. The first oxidation (nitritation, consumption of ammonia) consumes alkalinity (Downing et al., 1964).

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 oxidize 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 $>50\,g\ CaCO_3\,m^{-3} = 1\,mol\ HCO_3\,m^{-3}$) has thus to be maintained to ensure a stable pH (Henze et al., 2000a).

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

Model limitations

Multi-step nitrification/denitrification. The simplified concept of one-step nitrification is sufficient for most municipal wastewater systems. However, the modeling 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 (Kaelin et al., 2009; Sin et al., 2008). These modeling objectives cannot be reached with any of the studied models, which consider nitrification and denitrification as a single step. 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 nitrification process are reported in Gujer (2010). Inhibitory effects are considered to be constant and are accounted for in the growth rate value (Henze et al., 2000a). This can cause calibration problems in case of variability of inhibitory compounds concentration in the influent or in the treatment plant. Some authors developed online respirometric methods to determine inhibition kinetics of nitrification (Kong et al., 1996; Nowak et al., 1995; Vanrolleghem et al., 1996).

OHO and ANO Decay

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

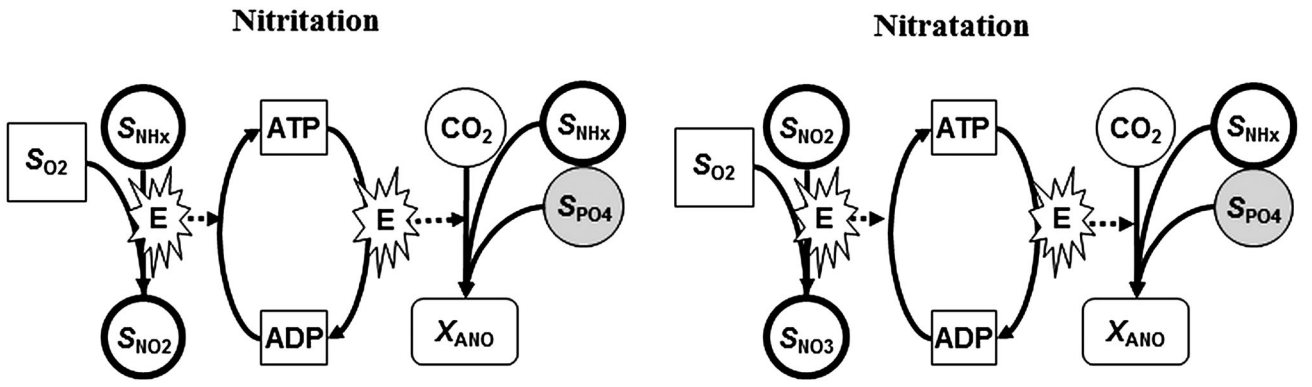


Figure 2. Simplified metabolism of autotrophic bacteria in 2 steps: nitritation and nitratation. Energy (*E*) is produced by oxidation of an electron donor (NH_X or NO_2), and then used for CO_2 uptake and growth.

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. 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 (Hao et al., 2010; van Loosdrecht and Henze, 1999): dormancy of bacteria, internal decay, external decay, and maintenance.

It should be noted that the anaerobic and anoxic conditions have been found to lower the OHO (Siegrist et al., 1999) and ANO (Munz et al., 2011; Siegrist et al., 1999) decay rates.

Modeling. Two concepts are used:

(1) The death-regeneration concept. Two sub-concepts have to be distinguished:
   (1a) Death-regeneration with a component-based model (ASM1, Barker & Dold);
   (1b) Death-regeneration with a fraction-based model (ASM2d, UCTPHO+).
(2) 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 hydrolyzed, and then used again for OHO growth. Consequently, ANO decay contributes to OHO growth.

This concept also allows modeling anaerobic decay and 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 Modeling under PAO Decay Section), and explains the fate of the OHO storage pool during OHO decay. This can be considered as endogenous respiration of the storage pool.

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 modeled 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 and to keep the model as simple as possible (Dold et al., 1980). However, under anaerobic or 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).

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 (modeled 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 2 or 3 steps are represented schematically in Table V. The use of PAOs to biologically remove phosphorus is named the enhanced biological phosphorus removal process (EBPR or BioP).

Metabolic model: To conceptualize BioP, the Delft University of Technology (TUD) group introduced a metabolic model that considers cell internal reactions (Smolders et al., 1994a,b). The cell internal concentrations of metabolites (NADH, acetyl-CoA, ATP, etc.) are considered to be in steady state conditions. Consequently, these components are not modeled, and only the overall stoichiometric reaction is formulated. This results in a model structure that is similar to the others.

PHA Storage

Knowledge. Under anaerobic conditions, in the presence of substrate, PAO store PHA. Figure 3 illustrates the main biochemical steps of PHA storage, that are described in more details below. Some experiments (Brdjanovic et al., 1998a; Comeau et al., 1987; Wentzel et al., 1989) 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 its counter-ions (Mg^{2+} and K^+) and protons allow the re-establishment of the proton motive force (Comeau et al., 1986). The polyP breakdown also provides most of the required energy to metabolize substrate into acetyl-CoA (Comeau et al., 1986) by phosphorylation of AMP into ADP (and later to ATP) (Wentzel et al., 1992).

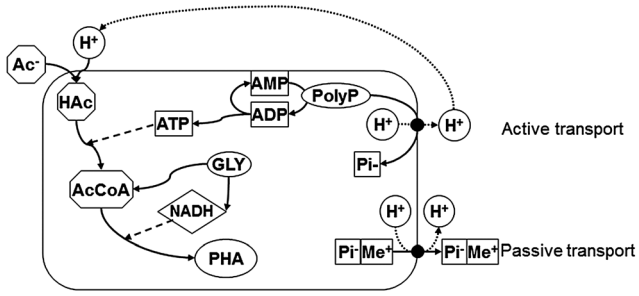
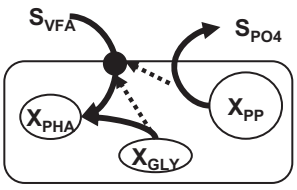
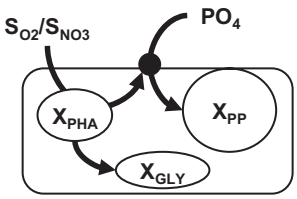
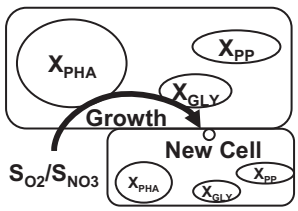


Figure 3. PHA storage: biochemical details (adapted from Wentzel et al., 2008) (Ac: acetate; HAc: protonated acetate, AcCoA: acetyl-CoA; ATP, ADP, AMP: Adenosine tri-(di-, mono-)phosphate; GLY: glycogen; Me: metal ion; NADH: Nicotinamide adenine dinucleotide; Pi: phosphorus ion; PHA: poly-β-hydroxyalkanoates; PolyP: polyphosphates).

Reducing power. Two theories for NADH production were developed (Jenkins and Tandoi, 1991; Wentzel et al., 1992): The "Comeau–Wentzel model" hypothesizes that NADH is provided by the anaerobic oxidation of acetate through the TCA cycle, whereas the "Mino model" considers that the NADH is provided by glycolysis under anaerobic conditions, turning stored glycogen into pyruvate and then into acetyl-CoA and CO_2 (Mino et al., 1998; Oehmen et al., 2007). This reaction also provides energy for acetate uptake and conversion to acetyl-CoA. The Mino model theory is well accepted and supported by experimental evidence, but the oxidative part of the TCA cycle seems to effectively supply part of the reducing power for PHA formation under certain conditions (Mino et al., 1998; Zhou et al., 2010). Oehmen et al. (2007) hypothesize that either each metabolic pathway is used by a specific microbial group of PAOs, or that PAOs are able to use different metabolic pathways depending on their internal or external conditions.

PolyP storage pool. Mino et al. (1985) and Wentzel et al. (1989) observed that not all the stored polyP can be

Table V. 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
		

degraded. They hypothesized that two different polyP molecular weights exist: short polyP chains have low molecular weight and can be released, whereas long polyP chains cannot. No experimental evidence has supported this hypothesis so far. Glycogen limitation, however, has been shown to result in the incomplete degradation of polyP (Brdjanovic et al., 1998c). Such a limitation may occur at a low pH (<7.3) in the presence of an excess of VFAs.

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

pH dependency. The energy requirements for S_{VFA} uptake increased with a higher pH, in order to maintain the proton motive force for S_{VFA} transport (Mino et al., 1998). This leads to an increased phosphorus release to S_{VFA} uptake ratio.

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). GAOs have thus 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 favored by higher temperatures and lower pH.

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

(1) Energy for storage is provided by polyP breakdown, and reducing power production is not considered (ASM2d, UCTPHO+, Barker & Dold, ASM3 + BioP).
(2) Energy is provided by polyP breakdown and glycogen degradation, while reducing power is also generated through glycolysis (ASM2d + TUD).

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 recognized in the form of a PHA formation yield. This causes a "COD loss," hypothesized to be H_2 formation for mathematical modeling (see Modeling under Fermentation Section).

Reducing power. In concept 1 the redox balance in the cell is neglected. In concept 2 NADH production comes from glycogen hydrolysis under anaerobic conditions; and under anoxic conditions NO_x utilization 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,b).

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.

Model limitations

Reducing power. In cases of glycogen depletion, the substrate storage may stop (Brdjanovic et al., 1998c), and models using the first concept (no 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) (see Knowledge under PHA Storage Section). 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).

PolyP Storage

Knowledge. In the presence of an electron acceptor and the absence of 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 phosphates and are able to store up to 12% of their dry weight as polyP granules (against 1–3% 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 (Mino et al., 1998; Smolders et al., 1994a).

Modeling. Models differ in the source of energy for polyP storage and in the overall concept for energy utilization:

(1) Growth and polyP storage processes are independent (ASM2d and ASM3 + BioP). Consequently, PHA oxidation is the result of phosphate uptake and growth.
(2) Storage pool restoration and growth are coupled (ASM2d + TUD). A part of the energy provided by PHA oxidation is allocated to each process.
(3) 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).

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 modeled 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.

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 (see Model Limitations under Ordinary Heterotrophic Organisms (OHO) Growth Section), 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 in Model Limitations under PolyP Storage Section 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 favored 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 (Barat et al., 2011; Maurer and Boller, 1999; Maurer and Gujer, 1998; Musvoto et al., 2000). In case of chemical phosphorus removal (by adding, e.g., iron, aluminum, or calcium salts) a chemical precipitation model also needs to be added.

PAO Growth

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 oxidized 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 behavior. In case of phosphate limitation, Wentzel et al. (1989) observed that growth continued and hypothesized 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 oxidize 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 subgroups of PAOs are capable to use only nitrite and other subgroups 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 depend on the PHA conversion rate and on the PHA storage capacity, provided that a sufficient minimum SRT is attained.

Modeling. Two main concepts are used in the five published BioP models:

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

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

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

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

Denitrification. PAO denitrification is considered in all studied BioP models. As a simplification, all models consider a single homogenous population. 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. This last concept is the one explicitly chosen in UCTPHO+. This way to model PAO denitrification has been successfully applied in several models, whereas the concept of two PAO populations leads systematically to the dominance of the aerobic PAOs (Hu et al., 2007). Table VI indicates whether the models use a different growth or polyP storage yield in aerobic and anoxic conditions.

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

Model limitations. The Barker & Dold model considers polyP with a high molecular weight. As already discussed in paragraph in Model Limitations under PHA Storage Section, 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 in Model Limitations under PolyP Storage Section.

Table VI. 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

PAO Decay

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. 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 (Lu et al., 2007; Siegrist et al., 1999). 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).

Modeling

Death-regeneration versus endogenous respiration. PAO decay is modeled according to the death-regeneration concept exclusively (ASM2d), as endogenous respiration exclusively (ASM3 + BioP and 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 VII synthesizes the concepts used in each model, depending on the electron acceptor conditions.

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, as 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 modeled to ensure that the storage products decay together with the biomass (ASM2d, ASM3 + BioP, Barker & Dold, UCTPHO+). 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 modeled 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 VIII synthesizes if the models 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 modeled with the aerobic and anoxic maintenance through PAO consumption.

Model limitations

Death-regeneration versus endogenous respiration. The limits highlighted for OHO and ANO decay processes (see paragraph in Model Limitations under OHO and ANO Decay Section) 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.

Table VII. Synthesis of decay concepts 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 VIII. 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	

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 & Dold and UCTPHO+ models will thus lead to an overestimation of the PAO decay, and to an underestimation of the biological phosphorus removal. Supressing 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).

Discussion

Diversity of Modeling Concepts and Their Theoretical Limitation

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 modelers. 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 IX (for OHO and ANO processes) and Table X (for PAO processes) synthesize all modeling concepts used in the seven studied models, for each standard process and the theoretical limitation they imply (in gray). For a complete description of the concepts and an explanation of the model's limitations, the reader is referred to the corresponding standard process paragraph.

Theoretical Limitations of Models

The main limitations of the models, highlighted in the "model limits" paragraph for each standard process, are synthesized in Tables IX and X (in gray) and may also be read and commented in a transversal way across the models.

State Variables and Substrates Considered Versus Characteristics of the Influent

Component-based models are more flexible, whereas fraction-based models are less complex. This should be considered depending on the influent type and variability.

A single particulate substrate is considered, which may be limiting in case of a peculiar influent.

None of the models consider simultaneously direct growth and growth on stored substrate, which will depend on the loading conditions (cyclic) that may vary.

Glycogen is neglected in all bioP models, however it may become limiting and neglecting it then leads to overestimation of substrate storage. This problem is overcome artificially in Barker & Dold model by introducing two polyP storage pools; one of them is not releasable during the substrate storage process.

Two processes may bias the substrate cycle in the system: OHO and ANO decay make the substrate available only for OHO, and PAO storage pool lysis in the UCTPHO+ model releases PHA in the form of XC_B.

The nutrients considered in the models differ: phosphorus is not a limiting nutrient in ASM1 and ASM3, whereas only Barker & Dold and UCTPHO+ models consider nitrates as possible nitrogen source for OHO and PAO growth, and polyP as possible phosphorus source for PAO growth. This should be taken into account in case of a peculiar influent or plant configuration that could lead to the depletion of one of these nutrients.

Simultaneous PAO growth and polyP storage accurately represent the interactions between metabolic mechanisms.

Modeling Concepts of Aerobic, Anoxic, and Anaerobic Processes Versus Plant Configuration

In models such as ASM1, ASM2d, ASM3 + BioP, or the Barker & Dold model, constant yields are considered to model some or all of the processes occurring under different electron acceptor conditions, which could impact the biomass production, the substrate and the electron acceptor consumption depending on the importance of the respective conditions in the plant configuration.

In the same way, fermentation is modeled as a simplified transformation process for all models except the Barker & Dold model that describes an anaerobic growth process and the corresponding biomass production due to fermentation. In case of a large anaerobic zone, this anaerobic growth may not be neglected and other models than the Barker & Dold model may then lead to an underestimation of the biomass production. However ASM3 + BioP neglects fermentation as it considers hydrolysis as the rate-limiting step. Model users should check if this hypothesis is verified in his/her plant configuration and influent characteristics.

Table IX. Synthesis of modeling concepts for each of the standard processes and models and their theoretical limitations (in gray) for OHOs and ANOs processes.

	ASM1	Barker & Dold	ASM2d/ASM2d + TUD	ASM3/ASM3 + BioP	UCTPHO+
Hydrolysis (see Hydrolysis of Particulate Substrate Section)	**One step hydrolysis** *Component-based model*	**One step hydrolysis** *Component-based model*	**One step hydrolysis** *Fraction-based model*	**One step hydrolysis** *Fraction-based model*	**Direct growth on particulate substrate**
	Anaerobic hydrolysis not considered Ammonification needed	Ammonification needed			Anaerobic hydrolysis not considered
	For all models: XC_B as only particulate fraction—Simplified mechanisms: chemical dissolution, mass transport, storage				
Fermentation (see Fermentation Section)	—	**Anaerobic growth process**	**Transformation**	—	**Transformation**
			Anaerobic biomass production neglected	**Neglected in ASM3 + BioP**	Anaerobic biomass production neglected
OHO growth (see Ordinary Heterotrophic Organisms (OHO) Growth Section)	**Direct growth on substrate**	**Direct growth on substrate**	**Direct growth on substrate**	**Storage—Growth**	**Direct growth on substrate**
	NH_X as only nitrogen source	*NH_X/NO_X as nitrogen source*	*NH_X as only nitrogen source*		*NH_X/NO_X as nitrogen source*
	P not limiting		Constant yields in anoxic condition	Poor predictions for non constant growth and storage rates	
	Constant yields in anoxic condition		NO_X not as N source	P not limiting	
	NO_X not as N source			Direct growth on S_B not considered	
				NO_X not as N source	
	For all models: Simplified mechanisms: one-step denitrification				
ANO Growth (see Autotrophic Nitrifying Organisms (ANO) Growth Section)	For all models: **One-step nitrification**				
	For all models: Simplified mechanisms: one-step nitrification and inhibitory effects not considered				
OHO and ANO decay (see OHO and ANO Decay Section)	**Death-regeneration**	**Death-regeneration**	**Death-regeneration**	**Endogenous respiration**	**Death-regeneration**
	Component-based model	*Component-based model*	*Fraction-based model*		*Fraction-based model*
	ASM1, Barker & Dold, ASM2d and ASM2+ TUD: Substrate available only for OHOs			Anaerobic decay not considered	Substrate available only for OHOs
	For all models: Simplified mechanisms: dormancy, maintenance, predation				

Table X. Synthesis of modeling concepts for each of the standard processes and models and their theoretical limitations (in gray) for PAOs processes.

	Barker & Dold	ASM2d	ASM3 + BioP	UCTPHO+	ASM2d + TUD
PHA storage (see Model Limitations Section in PHA Storage)	**Energy from polyP, reducing power neglected**	**Energy from polyP, reducing power neglected**	**Energy from polyP, reducing power neglected**	**Energy from polyP, reducing power neglected**	**Energy from polyP, reducing power from glycogen**
	Glycogen neglected	Glycogen neglected	Fermentation neglected Glycogen neglected	Glycogen neglected	
	For all models: Simplified mechanisms: competition with GAOs and effect of pH on acetate uptake (except **ASM2d + TUD**)				
PolyP storage (see Model Limitations Section in PolyP Storage)	**Simultaneous growth and polyP storage**	**Uncoupled processes**	**Uncoupled processes**	**Simultaneous growth and polyP storage**	**Coupled processes**
		Constant yields	Constant yields		
PAO growth (see Model Limitations under PAO Growth Section)		**Growth**	**Growth**		**Growth**
	Two polyP storage pools	Growth and polyP storage not linked	Growth and polyP storage not linked		NO_X not as N source and polyp not as P source
	Constant yield	NO_X not as N source and PolyP not as P source Constant yield	NO_X not as N source and PolyP not as P source		
	For all models: Biologically induced phosphate precipitation				
	For all models: Direct growth on S_{VFA} and S_F not considered under aerobic conditions				
PAO decay (see Model Limitations under PAO Decay Section)	**Endogenous respiration Maintenance**	**Death-regeneration**	**Endogenous respiration**	**Endogenous respiration Maintenance**	**Endogenous respiration Maintenance**
	Constant rates under anoxic and anaerobic conditions		Anaerobic decay and anaerobic maintenance not modeled	Constant rate under anoxic and anaerobic conditions	
	For all models: Aerobic maintenance not considered				
	For all models: PHA and glycogen utilization for maintenance are not considered				
PAO storage pool lysis (see Model Limitations under PAO Decay Section)	**PolyP lysis**	**PHA lysis**	**Respiration of stored compounds**	**PolyP lysis**	
	PHA lysis		**PolyP lysis**	**PHA lysis**	
			Electron acceptor consumed	PHA released in the form of XC_B	Storage pool lysis not modeled

Death-regeneration is simpler and adequate under anaerobic conditions, while endogenous respiration is closer to reality and applicable for secondary substrate use. One or the other concept should be preferred depending on the modeling project.

BioP models neglect aerobic maintenance and the ASM3 + BioP model does not model anaerobic decay and maintenance, which could lead to overestimating the PAO growth. They neither consider PHA and glycogen utilization for maintenance processes.

Two of the seven models, ASM1 and UCTPHO+, do not consider anaerobic hydrolysis, which could cause limitation in the use of these models, especially in case of UCTPHO+ as substrate may not be available for PHA storage when needed.

Other Simplifications of the Models That may Limit Their Use

In all considered models enitrification and nitrification are modeled as one-step, which is not suitable to predict nitrite accumulation or N_2O production, for example.

Phenomena such as dormancy of bacteria, maintenance or predation are lumped into decay processes, in the same way that mechanisms such as chemical dissolution, mass transport or storage that make slowly biodegradable substrate available for bacteria growth with a certain delay, are lumped into a single hydrolysis process. Consequently, if some of these aspects need to be described and modeled more precisely within the framework of a particular study, the remaining processes would be seen with a different significance, which would imply different parameter values.

Two processes that may interfere with BioP processes are not considered in these models: the competition between PAO and GAO for substrate, and the biologically induced phosphate precipitation. These processes may have a significance impact on phosphorus removal depending on the influent characteristics and on the plant.

Choice of Model

The model limitations highlighted in the previous paragraph should help model users to choose a model adequate to the modeling objectives and to the environmental conditions of the WWTP to be modeled.

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

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

Existing Model Modifications

Once the model is chosen, the user may have to include some modifications, either to reach the modeling 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. (2010), 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 in Modeling under Hydrolysis of Particulate Substrate Section). Consequently, model users should be very careful in using default model parameters in modified models.

Increased Knowledge Needed

This review of biochemical knowledge on biological processes and the comparison of the different modeling 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 summarized in Table XI.

In addition, two other processes not included in these seven models could be mentioned for further research to predict more accurately wastewater treatment process:

- phosphorus precipitation (only considered in ASM2d but not be discussed in this article) to integrate the

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

Needs in research	Modeling process affected	Current modeling concept or solution	Modeling problem	Research needs in practice and in understanding
OHO and ANO processes				
Predation (e.g., protozoa activity, bacteriophages)	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 modeling output is likely small	**Needed for better understanding** the preference of polyphosphate or glycogen for anaerobic maintenance processes should be elucidated
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

phenomenon of biologically induced phosphorus precipitation, which requires to model pH and other ions such as carbonate and magnesium (Barat et al., 2011; Maurer and Boller, 1999; Maurer and Gujer, 1998),
– filamentous organisms growth that can affect settling (Kappeler and Gujer, 1994a,b; Martins et al., 2004).

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 modeling concepts. A schematic representation has been developed and applied to the modeling concepts for each standard process as an additional visualization to complement the well-known Gujer matrix notation.

First, this representation will help model users to better understand modeling 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 (modeling schools), and highlights their main theoretical limits that should be taken into account when selecting a model in a modeling 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 modeled 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,
– Modeling glycogen adds model complexity but also completeness and
– Simultaneous PAO growth and polyP storage accurately represent the interactions between metabolic mechanisms.

However, the consequences of using one model over another depend on the wastewater treatment plant under study. Consequently, future work should involve a comparison of the results obtained with each of these 7 models for some wastewater treatment plants chosen with different configurations and influent in order to evaluate the effects of modeling different processes.

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.

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
o-PO4	orthophosphate ions (H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, or PO_4^{3-})
OHO	ordinary heterotrophic organisms
Ox	oxic (aerobic) conditions
PAO	phosphorus accumulating organisms
PHA	poly-β-hydroxyalkanoates
PHB	poly-β-hydroxybutyrate
PHV	poly-β-hydroxyvalerate
PolyP	polyphosphates
TCA	tricarboxylic acid (cycle)
ThOD	theoretical oxygen demand
TSS	total suspended solids
VFA	volatile fatty acids
VSS	volatile suspended solids
WWTP	wastewater treatment plant

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, Irstea, EnviroSim Associates Ltd., Kurita Water Industries Ltd., TU Vienna, BOKU Vienna, 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 was a Ph.D. student at Irstea (former Cemagref) (France) and modelEAU (Université Laval, Québec, Canada).

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

References

Barat R, Montoya T, Seco A, Ferrer J. 2011. Modelling biological and chemically induced precipitation of calcium phosphate in enhanced biological phosphorus removal systems. Water Res 45(12):3744–3752.

Barker PS, Dold PL. 1995. COD and nitrogen mass balances in activated sludge systems. Water Res 29(2):633–643.

Barker PS, Dold PL. 1996. Denitrification behaviour in biological excess phosphorus removal activated sludge systems. Water Res 30(4):769–780.

Barker PS, Dold PL. 1997. General model for biological nutrient removal activated-sludge systems: Model presentation. Water Environ Res 69(5):969–984.

Beccari M, Dionisi D, Giuliani A, Majone M, Ramadori R. 2002. Effect of different carbon sources on aerobic storage by activated sludge. Water Sci Technol 45(6):157–168.

Beun JJ, Dircks K, van Loosdrecht MCM, Heijnen JJ. 2002. Poly-beta-hydroxybutyrate metabolism in dynamically fed mixed microbial cultures. Water Res 36(5):1167–1180.

Brdjanovic D, Slamet A, van Loosdrecht MCM, Hooijmans CM, Alaerts GJ, Heijnen JJ. 1998a. Impact of excessive aeration on biological phosphorus removal from wastewater. Water Res 32(1):200–208.

Brdjanovic D, van Loosdrecht MCM, Hooijmans CM, Alaerts GJ, Heijnen JJ. 1998b. Minimal aerobic sludge retention time in biological phosphorus removal systems. Biotechnol Bioeng 60(3):326–332.

Brdjanovic D, van Loosdrecht MCM, Hooijmans CM, Mino T, Alaerts GJ, Heijnen JJ. 1998c. Bioassay for glycogen determination in biological phosphorus removal systems. Water Sci Technol 37(4–5):541–547.

Buchan L. 1983. Possible biological mechanism of phosphorus removal. Water Sci Technol 15(3–4):87–103.

Comeau Y, Takács I. Schematic representation of activated sludge models; Water Environment Federation 81st Annual Technical Exhibition & Conference October 18–22, 2008; Chicago, IL, USA.

Comeau Y, Hall KJ, Hancock REW, Oldham WK. 1986. Biochemical model for enhanced biological phosphorus removal. Water Res 20(12):1511–1521.

Comeau Y, Rabinowitz B, Hall KJ, Oldham WK. 1987. Phosphate release and uptake in enhanced biological phosphorus removal from wastewater. J Water Pollut Control Fed 59(7):707–715.

Corominas L, Rieger L, Takács I, Ekama G, Hauduc H, Vanrolleghem PA, Oehmen A, Gernaey KV, Comeau Y. 2010. New framework for standardized notation in wastewater treatment modelling. Water Sci Technol 61(4):841–857.

Curds CR. 1971. Computer simulations of microbial population dynamics in the activated-sludge process. Water Res 5(11):1049–1066.

de Kreuk MK, Kishida N, Tsuneda S, van Loosdrecht MCM. 2010. Behavior of polymeric substrates in an aerobic granular sludge system. Water Res 44(20):5929–5938.

Dold PL, Ekama GA, Marais GvR. 1980. A general model for the activated sludge process. Prog Water Technol 12(6):47–77.

Downing AA, Painter HA, Knowles G. 1964. Nitrification in the activated-sludge process. J Inst Sewage Purif 64(2):130–158.

Ekama GA, Marais GvR. 1979. Dynamic behavior of the activated sludge process. J Water Pollut Control Fed 51(3I):534–556.

Ekama GA, Wentzel MC. 1999. Difficulties and developments in biological nutrient removal technology and modelling. Water Sci Technol 39(6):1–11.

Ekama GA, Wentzel MC. 2004. Modelling inorganic material in activated sludge systems. Water SA 30(2):153–174.

Gernaey KV, van Loosdrecht MCM, Henze M, Lind M, Jørgensen SB. 2004. Activated sludge wastewater treatment plant modelling and simulation: State of the art. Environ Model Softw 19(9):763–783.

Goel R, Mino T, Satoh H, Matsuo T. 1999. Modeling hydrolysis processes considering intracellular storage. Water Sci Technol 39(1):97–105.

Grady CPL, Gujer W, Henze M. 1986. A model for single-sludge wastewater treatment systems. Water Sci Technol 18(6):47–61.

Guisasola A, Sin G, Baeza JA, Carrera J, Vanrolleghem PA. 2005. Limitations of ASM1 and ASM S3: A comparison based on batch oxygen uptake rate profiles from different full-scale wastewater treatment plants. Water Sci Technol 52(10–11):69–77.

Gujer W. 2010. Nitrification and me—A subjective review. Water Res 44(1):1–19.

Gujer W, Larsen TA. 1995. The implementation of biokinetics and conservation principles in ASIM. Water Sci Technol 31(2):257–266.

Gujer W, Henze M, Mino T, van Loosdrecht MCM. 1999. Activated sludge model no. 3. Water Sci Technol 39(1):183–193.

Gujer W, Henze M, Mino T, van Loosdrecht MCM. 2000. Activated sludge model no. 3. In: Henze M, Gujer W, Van Loosdrecht M, Mino T, editors. Activated sludge models ASM1, ASM2, ASM2d and ASM3. London, UK: IWA Publishing.

Hao X, Wang Q, Zhang X, Cao Y, Mark Loosdrecht CMv. 2009. Experimental evaluation of decrease in bacterial activity due to cell death and activity decay in activated sludge. Water Res 43(14):3604–3612.

Hao XD, Wang QL, Zhu JY, van Loosdrecht MCM. 2010. Microbiological endogenous processes in biological wastewater treatment systems. Crit Rev Environ Sci Technol 40(3):239–265.

Hauduc H, Gillot S, Rieger L, Ohtsuki T, Shaw A, Takács I, Winkler S. 2009. Activated sludge modelling in practice—An international survey. Water Sci Technol 60(8):1943–1951.

Hauduc H, Rieger L, Takács I, Heduit A, Vanrolleghem PA, Gillot S. 2010. A systematic approach for model verification: Application on seven published activated sludge models. Water Sci Technol 61(4):825–839.

Hauduc H, Rieger L, Ohtsuki T, Shaw A, Takács I, Winkler S, Heduit A, Vanrolleghem PA, Gillot S. 2011. Activated sludge modelling: Development and potential use of a practical applications database. Water Sci Technol 63(10):2164–2182.

Henze M, Grady CPL, Gujer W, Marais GvR, Matsuo T. 1987. Activated sludge model no. 1. London, UK: IAWPRC Publishing.

Henze M, Gujer W, Mino T, Matsuo T, Wentzel MC, Marais GvR, van Loosdrecht MCM. 1999. Activated sludge model no. 2d, ASM2d. Water Sci Technol 39(1):165–182.

Henze M, Grady CPL, Gujer W, Marais GvR, Matsuo T. 2000a. Activated sludge model no. 1. In: Henze M, Gujer W, van Loosdrecht M, Mino T, editors. Activated sludge models ASM1, ASM2, ASM2d and ASM3. London, UK: IWA Publishing.

Henze M, Gujer W, Mino T, Matsuo T, Wentzel MC, Marais GvR, van Loosdrecht MCM. 2000b. Activated sludge model no. 2d. In: Henze M, Gujer W, Van Loosdrecht M, Mino T, editors. Activated sludge models ASM1, ASM2, ASM2d and ASM3. London, UK: IWA Publishing.

Hiatt WC, Grady CPL. 2008. An updated process model for carbon oxidation, nitrification, and denitrification. Water Environ Res 80(11):2145–2156.

Hu ZR, Wentzel MC, Ekama GA. 2007. A general kinetic model for biological nutrient removal activated sludge systems: Model development. Biotechnol Bioeng 98(6):1242–1258.

Jenkins D, Tandoi V. 1991. The applied microbiology of enhanced biological phosphate removal accomplishments and needs. Water Res 25(12):1471–1478.

Kaelin D, Manser R, Rieger L, Eugster J, Rottermann K, Siegrist H. 2009. Extension of ASM3 for two-step nitrification and denitrification and its calibration and validation with batch tests and pilot scale data. Water Res 43(6):1680–1692.

Kappeler J, Gujer W. 1994a. Development of a mathematical-model for aerobic bulking. Water Res 28(2):303–310.

Kappeler J, Gujer W. 1994b. Scumming due to actinomycetes—Towards a better understanding by modeling. Water Res 28(4):763–779.

Karahan-Gul O, van Loosdrecht MCM, Orhon D. 2003. Modification of activated sludge model no. 3 considering direct growth on primary substrate. Water Sci Technol 47(11):219–225.

Kong Z, Vanrolleghem PA, Willems P, Verstraete W. 1996. determination of inhibition kinetics of carbon oxidation and nitrification with a respirometer. Water Res 30:825–836.

Kraemer J, Constantine T, Crawford G, Erdal Z, Katehis D, Johnson B, Daigger G. Process design implications due to differences in anaerobic COD stabilisation models;81st Annual Water Environment Federation Technical Exhibition and Conference October 18-22, 2008; Chicago, Illinois, USA. Water Environment Federation.

Krishna C, van Loosdrecht MCM. 1999. Substrate flux into storage and growth in relation to activated sludge modeling. Water Res 33(14):3149–3161.

Larrea L, Irizar I, Hildago ME. 2002. Improving the predictions of ASM2d through modelling in practice. Water Sci Technol 45(6):199–208.

Lijklema L. 1973. Model for nitrification in activated sludge process. Environ Sci Technol 7(5):428–433.
Lopez C, Pons MN, Morgenroth E. 2006. Endogenous processes during long-term starvation in activated sludge performing enhanced biological phosphorus removal. Water Res 40(8):1519–1530.
Lopez-Vazquez CM, Oehmen A, Hooijmans CM, Brdjanovic D, Gijzen HJ, Yuan Z, van Loosdrecht MCM. 2009. Modeling the PAO-GAO competition: Effects of carbon source, pH and temperature. Water Res 43(2):450–462.
Lu H, Keller J, Yuan Z. 2007. Endogenous metabolism of Candidatus accumulibacter phosphatis under various starvation conditions. Water Res 41(20):4646–4656.
Martins AMP, Picioreanu C, Heijnen JJ, van Loosdrecht MCM. 2004. Three-dimensional dual-morphotype species modeling of activated sludge flocs. Environ Sci Technol 38(21):5632–5641.
Maurer M, Boller M. 1999. Modelling of phosphorus precipitation in wastewater treatment plants with enhanced biological phosphorus removal. Water Sci Technol 39(1):147–163.
Maurer M, Gujer W. 1998. Dynamic modelling of enhanced biological phosphorus and nitrogen removal in activated sludge systems. Water Sci Technol 38(1):203–210.
Meijer SCF. 2004. Theoretical and practical aspects of modelling activated sludge processes. Delft, The Netherlands: Delft University of Technology. 218 p.
Mino T, Kawakami T, Matsuo T. 1985. Location of phosphorus in activated sludge and function of intracellular polyphosphates in biological phosphorus removal process. Water Sci Technol 17(2-3-3 pt 1):93–106.
Mino T, van Loosdrecht MCM, Heijnen JJ. 1998. Microbiology and biochemistry of the enhanced biological phosphate removal process. Water Res 32(11):3193–3207.
Morgenroth E, Kommedal R, Harremoes P. 2002. Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment—A review. Water Sci Technol 45(6):25–40.
Moussa MS, Hooijmans CM, Lubberding HJ, Gijzen HJ, van Loosdrecht MCM. 2005. Modelling nitrification, heterotrophic growth and predation in activated sludge. Water Res 39(20):5080–5098.
Muller A, Wentzel MC, Loewenthal RE, Ekama GA. 2003. Heterotroph anoxic yield in anoxic aerobic activated sludge systems treating municipal wastewater. Water Res 37(10):2435–2441.
Munz G, Lubello C, Oleszkiewicz JA. 2011. Modeling the decay of ammonium oxidizing bacteria. Water Res 45:557–564.
Musvoto E, Wentzel M, Loewenthal R, Ekama G. 2000. Integrated chemical-physical processes modelling—I. Development of a kinetic-based model for mixed weak acid/base systems. Water Res 34(6):1857–1867.
Ni BJ, Rittmann BE, Yu HQ. 2010. Modeling predation processes in activated sludge. Biotechnol Bioeng 105(6):1021–1030.
Nowak O, Svardal K, Schweighofer P. 1995. The dynamic behaviour of nitrifying activated sludge systems influenced by inhibiting wastewater compounds. Water Sci Technol 31(2):115–124.
Nowak O, Svardal K, Franz A, Kuhn V. 1999. Degradation of particulate organic matter—A comparison of different model concepts. Water Sci Technol 39(1):119–127.
Oehmen A, Lemos PC, Carvalho G, Yuan Z, Keller J, Blackall LL, Reis MAM. 2007. Advances in enhanced biological phosphorus removal: From micro to macro scale. Water Res 41(11):2271–2300.
Oehmen A, Lopez-Vazquez CM, Carvalho G, Reis MAM, van Loosdrecht MCM. 2010. Modelling the population dynamics and metabolic diversity of organisms relevant in anaerobic/anoxic/aerobic enhanced biological phosphorus removal processes. Water Res 44(15):4473–4486.
Orhon D, Sozen S, Artan N. 1996. The effect of heterotrophic yield on the assessment of the correction factor for anoxic growth. Water Sci Technol 34(5–6):67–74.
Orhon D, Cokgor EU, Sozen S. 1998. Dual hydrolysis model of the slowly biodegradable substrate in activated sludge systems. Biotechnol Tech 12(10):737–741.
Refsgaard JC, van der Sluijs JP, Højberg AL, Vanrolleghem PA. 2007. Uncertainty in the environmental modelling process—A framework and guidance. Environ Model Softw 22(11):1543–1556.
Rieger L, Koch G, Kuhni M, Gujer W, Siegrist H. 2001. The EAWAG Bio-P module for activated sludge model no. 3. Water Res 35(16):3887–3903.
Serralta J, Ferrer J, Borras L, Seco A. 2004. An extension of ASM2d including pH calculation. Water Res 38(19):4029–4038.
Siegrist H, Brunner I, Koch G, Phan LC, Van Le C. 1999. Reduction of biomass decay rate under anoxic and anaerobic conditions. Water Sci Technol 39(1):129–137.
Sin G, Guisasola A, De Pauw DJW, Baeza JA, Carrera J, Vanrolleghem PA. 2005. A new approach for modelling simultaneous storage and growth processes for activated sludge systems under aerobic conditions. Biotechnol Bioeng 92(5):600–613.
Sin G, Kaelin D, Kampschreur MJ, Takács I, Wett B, Gernaey KV, Rieger L, Siegrist H, van Loosdrecht MCM. 2008. Modelling nitrite in wastewater treatment systems: A discussion of different modelling concepts. Water Sci Technol 58(6):1155–1171.
Smolders GJF, van Der Meij J, van Loosdrecht MCM, Heijen JJ. 1994a. Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process. Biotechnol Bioeng 44(7):837–848.
Smolders GJF, van der Meij J, van Loosdrecht MCM, Heijnen JJ. 1994b. Model of the anaerobic metabolism of the biological phosphorus removal process: Stoichiometry and pH influence. Biotechnol Bioeng 43(6):461–470.
Sozen S, Cokgor EU, Orhon D, Henze M. 1998. Respirometric analysis of activated sludge behaviour—II. Heterotrophic growth under aerobic and anoxic conditions. Water Res 32(2):476–488.
Sudiana IM, Mino T, Satoh H, Nakamura K, Matsuo T. 1999. Metabolism of enhanced biological phosphorus removal and non-enhanced biological phosphorus removal sludge with acetate and glucose as carbon source. Water Sci Technol 39(6):29–35.
Takács I. 2005. The Petersen/Petersen a.k.a. Gujer matrix—Revisiting tha activated sludge model. Water 21, IWA Publishing, February 2005, p51.
Takács I, Dold PL, Morgenroth E, Boltz J, Schraa O, Belia E, Shaw A, Wett B, Jones R, Comeau Y. 2011. Structured process models for nutrient removal. Chapter 10. In: Nutrient removal—Manual of practice no. 34. Alexandria, VA, USA: Water Environment Federation, McGraw-Hill. p 356–486.
van Aalst-Van Leeuwen MA, Pot MA, van Loosdrecht MCM, Heijnen JJ. 1997. Kinetic modeling of poly(Î²-hydroxybutyrate) production and consumption by *Paracoccus pantotrophus* under dynamic substrate supply. Biotechnol Bioeng 55(5):773–782.
van Loosdrecht MCM, Heijnen JJ. 2002. Modelling of activated sludge processes with structured biomass. Water Sci Technol 45(6):13–23.
van Loosdrecht MCM, Henze M. 1999. Maintenance, endogeneous respiration, lysis, decay and predation. Water Sci Technol 39(1):107–117.
van Loosdrecht MCM, Hooijmans CM, Brdjanovic D, Heijnen JJ. 1997a. Biological phosphate removal processes. Appl Microbiol Biotechnol 48(3):289–296.
van Loosdrecht MCM, Pot MA, Heijnen JJ. 1997b. Importance of bacterial storage polymers in bioprocesses. Water Sci Technol 35(1):41–47.
Vanrolleghem PA, Kong Z, Coen F. 1996. Full-scale on-line assessment of toxic wastewaters causing change in biodegradation model structure and parameters. Water Sci Technol 33(2):163–175.
Warner APC, Ekama GA, Marais GvR. 1986. The activated sludge process. IV. Application of the general kinetic model to anoxic–aerobic digestion of waste activated sludge. Water Res 20(8):943–958.
Wentzel MC, Ekama GA, Loewenthal RE, Dold PL, Marais GvR. 1989. Enhanced polyphosphate organism cultures in activated sludge systems. Part II: Experimental behaviour. Water SA 15(2):71–88.
Wentzel MC, Ekama GA, Marais GvR. 1992. Processes and modelling of nitrification denitrification biological excess phosphorus removal systems—A review. Water Sci Technol 25(6):59–82.
Wentzel MC, Comeau Y, Ekama G, van Loosdrecht MCM, Brdjanovic D. 2008. ''Enhanced biological phosphorus removal'' in biological wastewater treatment - principles, modelling and design. London, UK: IWA Publishing.
Zhou Y, Pijuan M, Oehmen A, Yuan Z. 2010. The source of reducing power in the anaerobic metabolism of polyphosphate accumulating organisms (PAOs)—A mini-review. Water Sci Technol 61(7):1653–1662.