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

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Abstract

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

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

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

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

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

NOMENCLATURE

An: Anaerobic conditions

ANO: Autotrophic nitrifying organisms

ASM: Activated sludge model

ATP: Adenosine triphosphate

Ax: Anoxic conditions

COD: Chemical oxygen demand

BioP: Biological phosphate removal

FADH: Flavin adenine dinucleotide

GAO: Glycogen accumulating organisms

ISS: Inorganic suspended solids

NADH: Nicotinamide adenine dinucleotide

OHO: Ordinary heterotrophic organisms

Ox: Oxic (aerobic) conditions

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

1. 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 modelling project, and ASM1 turns out too 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. Each row of this matrix stands for a process and each column for a state variable of the model. The

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

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

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

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

111 ## 2. METHODOLOGY

112 ### 2.1. Studied models

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

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

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

131 **2.2. Model processes**

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

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

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

140 **2.3. A new schematic representation**

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

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

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

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

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

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

173 ### 2.4. Literature review on theoretical knowledge vs modelling concepts

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

180 ## 3. RESULTS

181 ### 3.1. General modelling concepts

182 #### *3.1.1. State variables*

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

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

3.1.2. Component-based / fraction-based models

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

3.1.2.1. Component-based model

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

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

3.1.2.2. Fraction-based model

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

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

3.1.3. Undegradable organics from influent and from biomass decay

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

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

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

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

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

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

TSS is not considered in this study.

3.1.6. Alkalinity

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

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

239 **3.2. OHO and ANO processes**

240 *3.2.1. Hydrolysis of particulate substrate*

241 3.2.1.1. Knowledge

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

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

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

255 Electron acceptor conditions. Hydrolytic enzyme synthesis depends on the electron acceptor
256 conditions, but their activity is not affected by the electron acceptor conditions (Goel *et al.*,
257 1999), which enables hydrolysis processes to continue under anoxic and anaerobic conditions.
258 Hydrolysis due to protozoa activity will however depend on oxic conditions.

3.2.1.2. Modelling

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

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

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

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

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

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

299 The Barker & Dold model introduces a hydrolysis yield in anoxic and anaerobic conditions
300 that allows modelling the experimentally observed "COD loss" (Barker and Dold, 1995).
301 Although this observation is not explained so far, the "loss" is modelled by H_2 gas formation
302 (Kraemer *et al.*, 2008). The S_{H2} state variable is thus added to the model to reach continuity
303 (Hauduc *et al.*, 2010b).

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

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

309 3.2.1.3. Model limitations

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

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

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

324 *3.2.2. Fermentation*

325 3.2.2.1. Knowledge

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

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

331 3.2.2.2. Modelling

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

333 – The first concept considers fermentation as a transformation (ASM2d, UCTPHO+)

334 – In the second concept fermentation is described as an anaerobic growth process.

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

337 Barker and Dold (1995) experimentally observed a COD "loss" during anaerobic processes,
338 which they linked to fermentation, anaerobic hydrolysis and S_{VFA} sequestration. This
339 phenomenon has been modelled by a S_{VFA} formation yield (Y_{fe}) in the fermentation process.
340 The loss of $(1-Y_{fe})$ g ThOD.g S_{VFA}^{-1} is modelled through H_2 gas formation by Kraemer *et al.*
341 (2008), and a S_{H2} state variable has thus been added to reach model continuity (Hauduc *et al.*,
342 2010b).

343 3.2.2.3. Model limitations

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

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

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

3.2.3. Ordinary heterotrophic organisms (OHO) growth

3.2.3.1. Knowledge

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

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

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

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

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

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

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

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

396 triphosphate, which transports energy in the cell) formation through oxidative
397 phosphorylation in the electron transport chain (lower ATP formation per NADH (Y_{NADH_ATP})
398 in metabolic models) when nitrate is the electron acceptor instead of oxygen (Beun *et al.*,
399 2002; Muller *et al.*, 2003).

400 3.2.3.2. Modelling

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

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

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

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

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

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

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

with S_{Sub} being the considered substrate.

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

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

3.2.3.3. Model limitations

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

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

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

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

474 *3.2.4. Autotrophic Nitrifying Organisms (ANO) growth*

475 3.2.4.1. Knowledge

476 ANO oxidise ammonia to produce the required energy for CO_2 uptake and growth. This
477 oxidation of ammonia is named nitrification. It includes two steps that involve two distinct
478 groups of autotrophic organisms: ammonia oxidisers and nitrite oxidisers (figure 3). In the
479 first step, nitritation, ammonia oxidisers produce energy required for their growth through
480 ammonia oxidation into nitrite. Then, in the nitratation step, nitrite oxidisers convert nitrite
481 into nitrate to produce energy. The first oxidation (nitritation) consumes alkalinity (Downing
482 *et al.*, 1964).

483 Because a lot of energy is required to reduce CO_2, autotrophic organisms have a lower growth
484 yield than OHOs. Furthermore, the nitrifiers, which are obligate aerobic organisms, have a
485 higher requirement of oxygen than heterotrophs for their growth: in addition to their needs in
486 electron acceptor for respiration, oxygen is used to oxidise ammonia. Therefore, to ensure

487 good nitrification, it is necessary to provide sufficient dissolved oxygen to the activated
488 sludge and to maintain a minimum SRT to avoid the wash out of nitrifiers (Downing *et al.*,
489 1964). Nitrification is also inhibited by a low pH and sufficient alkalinity concentration
490 (generally >50 g $CaCO_3$. m^{-3} = 1 mol HCO_3^- m^{-3}) has thus to be maintained to ensure a stable
491 pH (Henze *et al.*, 2000a).

492 3.2.4.2. Modelling

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

496 3.2.4.3. Model limitations

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

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

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

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

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

520 *3.2.5. OHO and ANO decay*

521 3.2.5.1. Knowledge

522 van Loosdrecht and Henze (1999) published a literature review on the theoretical knowledge
523 regarding maintenance, endogenous respiration, lysis, decay and predation. Oxygen
524 consumption linked to a loss of biomass was observed by various authors since the end of the
525 19th century. This phenomenon has been explained by the concept of "endogenous
526 respiration" during which bacteria use their own storage pools of organic matter for
527 maintenance purposes instead of using external substrate. Other experiments have shown
528 accumulation of undegradable matter in the absence of substrate, leading to the cryptic growth
529 (growth on dead bacteria) or the "death-regeneration" concepts (van Loosdrecht and Henze,
530 1999).

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

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

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

3.2.5.2. Modelling

Two concepts are used (Table 10):

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

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

556 Death-regeneration concept. The biomass decay results in the release of a fraction (1-
557 $f_{XU\_OHO,lys}$) of particulate substrate and a fraction $f_{XU\_OHO,lys}$ of undegradable material. The
558 released particulate substrate will be hydrolysed, and then used again for OHO growth.
559 Consequently, ANO decay contributes to OHO growth.

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

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

573 3.2.5.3. Model limitations

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

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

584 Predation. Predation is explicitly modelled by Curds (1971), Lijklema (1973), Moussa *et al.*
585 (2005) and more recently by Ni et al (2010), considering a reduction of the active biomass
586 through protozoa consumption, their concentration being between 5% and 10% of the
587 MLVSS according to Curds (1971). Not considering predation may lead to variable kinetic
588 parameter values depending on the WWTP conditions.

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

600 **3.3. Biological phosphorus removal**

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

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

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

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

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

625 *3.3.1. PHA storage*

626 3.3.1.1. Knowledge

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

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

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

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

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

645 and CO_2 (Mino *et al.*, 1998; Oehmen *et al.*, 2007). This reaction also provides energy
646 for acetate uptake and conversion to acetyl-CoA.

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

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

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

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

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

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

3.3.1.2. Modelling

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

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

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

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

693 Reducing power. In the first concept the redox balance in the cell is neglected. In the second
694 concept, NADH production comes from glycogen hydrolysis under anaerobic conditions; and
695 under anoxic conditions NOx utilisation as electron acceptor in the oxidative phosphorylation
696 pathway stimulates the TCA cycle that produces NADH/FADH. The aerobic/anoxic
697 stoichiometry of ASM2d+TUD is dependent on 3 metabolic yields: ATP formation per
698 NADH (Y_{NADH_ATP}), biomass production per ATP ($Y_{ATP\_X,Bio}$) and NADH requirement for
699 PO_4 transport across the cell membreane (Y_{NADH_P}) (Smolders *et al.*, 1994a; Smolders *et al.*,
700 1994b).

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

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

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

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

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

3.3.1.3. Model limitations

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

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

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

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

3.3.2. PolyP storage

3.3.2.1. Knowledge

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

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

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

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

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

3.3.2.2. Modelling

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

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

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

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

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

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

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

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

3.3.2.3. Model limitations

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

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

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

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

811 *3.3.3. PAO growth*

812 3.3.3.1. Knowledge

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

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

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

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

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

837 3.3.3.2. Modelling

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

839 – In the first concept, PAO growth is similar to OHO growth and the process is
840 separated from polyP storage (ASM2d, ASM3+BioP, ASM2d+TUD).

841 – In the second concept, followed by UCTPHO+ and Barker & Dold, phosphate uptake
842 is simultaneous to growth: PAOs take up phosphate as nutrient for growth and store it
843 as energy source. Barker & Dold consider two polyP storage pools (low and high
844 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 15 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 normalised to PHA, and the storage pool restoration concept (3.3.2.2).

865 3.3.3.3. Model limitations

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

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

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

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

882 *3.3.4. PAO decay*

883 3.3.4.1. Knowledge

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

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

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

897 – Under aerobic conditions, PAOs use PHA, then glycogen (Brdjanovic *et al.*, 1998a;
898 Lopez *et al.*, 2006; Lu *et al.*, 2007), but seem not able to use polyP for energy
899 production (Lu *et al.*, 2007)
900 – Under anoxic conditions, PAOs use first PHA, which is rapidly depleted (Lopez *et al.*,
901 2006), then glycogen and polyP (Lu *et al.*, 2007). Experiments by Wentzel *et al.*
902 (1989) showed the so-called secondary P-release during endogenous mass loss, due to
903 polyP use.
904 – Under anaerobic conditions, PAOs would use both glycogen and polyP for
905 maintenance (Lopez *et al.*, 2006; Lu *et al.*, 2007).

906 3.3.4.2. Modelling

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

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

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

918 Undegradable particulate matter production. Only ASM2d+TUD does not consider
919 undegradable particulate matter production in the PAO decay process, because it is considered
920 that insufficient experimental proof was available to evaluate this released material (Meijer,
921 2004).

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

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

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

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

940 3.3.4.3. Model limitations

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

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

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

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

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

961 ## 4. DISCUSSION

962 ### 4.1. Diversity of modelling concepts

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

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

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

4.2. Theoretical limitations of models and choice of model

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

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

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

4.3. Existing model modifications

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

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

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

4.4. Increased knowledge needed

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

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

5. CONCLUSION

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

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

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

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

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

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

1048 treatment process, especially in the hydrolysis and decay processes, the role and importance
1049 of substrate storage by OHO and the multiple-step nitrification-denitrification processes.
1050 Concerning PAO processes, the competition between PAO and GAO is not fully understood,
1051 as is the use of stored compounds for maintenance and the role of the TCA cycle in the
1052 anaerobic PAO metabolism.

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8. CAPTIONS

Table 1. List of studied activated sludge models

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Table 13. PolyP storage process concept

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Table
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Table 1. List of studied activated sludge models

Models	Reference	Substrates	# of processes	# of state variables	# of interacting processes vs. variables	# of parameters														
						Total	Composition matrix parameters	Temperature adjustment	stoichiometry					kinetic					
									Hydrolysis	OHO	ANO	PAO	Biomass general	Hydrolysis	OHO	ANO	PAO	Biomass general
ASM1	Henze *et al.*, 2000a[(1)]	CN	**8**	**13**	**31**	**26**	2	7	-	1	1	-	1	3	6	5	-	-
Barker&Dold	Barker and Dold, 1997	CNP	**36**	**19**	**153**	**81**	16	18	2	5	2	8	-	4	9	5	11	1
ASM2d	Henze *et al.*, 2000b[(2)]	CNP	**21**	**19**	**136**	**74**	13	12	1	1	1	3	1	6	12	6	18	-
ASM3	Gujer *et al.*, 2000[(3)]	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	-

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

Table
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Table 2. List of processes and models considered

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

Table
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Table 3. Symbols used for kinetic expressions: examples

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

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

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

Description	Notation	Unit	ASM1			ASM2d			ASM3			ASM3 + BioP			Barker & Dold			UCT PHO+			ASM2d+ TUD		
			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						XX			XXX											
Fermentable organic matter	S_F	g COD.m^{-3}				XXX									X			XXX			XXX		
Fermentation product (volatile fatty acids)	S_{VFA}	g COD.m^{-3}				X									X			X			X		
Soluble undegradable organics	S_U	g COD.m^{-3}	X			XXX			XX			XXX			X			XXX			XXX		
Dissolved oxygen	S_{O2}	- g COD.m^{-3}	X			X			X			X			X			X			X		
COD particulate and colloidal																							
Particulate and colloidal biodeg. organics	XC_B	g COD.m^{-3}	X			XXX			XX			XXX			X			XXX			XXX		
Adsorbed slowly biodegradable substrate	X_{Ads}	g COD.m^{-3}																XXX					
Particulate undegradable organics	X_U	g COD.m^{-3}				XXX			XX			XXX									XXX		
Particulate undeg. organics from influent	$X_{U,Inf}$	g COD.m^{-3}	X												X			XXX					
Particulate undeg. endogenous products	$X_{U,E}$	g COD.m^{-3}	XX												XXX			XXX					
Nitrogen (N) and Phosphorus (P)																							
Ammonium and ammonia nitrogen	S_{NHx}	g N.m^{-3}		X			X			X			X			X			X			X	
Nitrate and nitrite	S_{NOx}	g N.m^{-3}		XX			XX			XX			XX			XX			XX			XX	
Dissolved nitrogen gas	S_{N2}	g N.m^{-3}					XX			XX			XX									XX	
Particulate and colloidal biodeg. organic N	$XC_{B,N}$	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_{PO4}	g P.m^{-3}					X						X				X		X			X	
Biomass																							
Ordinary heterotrophic organisms	X_{OHO}	g COD.m^{-3}	X			X			X			X			X			X			XX		
Autotrophic nitrifying organisms	X_{ANO}	g COD.m^{-3}	X			X			X			X			X			X			XX		
Phosphorus accumulating organisms	X_{PAO}	g COD.m^{-3}				X						X			X			X			XX		
Internal cells products																							
Storage compound in OHOs	$X_{OHO,Stor}$	g COD.m^{-3}							X			X											
Storage compound in PAOs	$X_{PAO,Stor}$	g COD.m^{-3}				X						X			X			X					
Stored poly-β-hydroxyalkanoates in PAOs	$X_{PAO,PHA}$	g COD.m^{-3}																			X		
Stored glycogen in PAOs	$X_{PAO,Gly}$	g COD.m^{-3}																			X		
Stored polyP in PAOs	$X_{PAO,PP}$	g P.m^{-3}					X						X						X			X	
Releasable stored polyP	$X_{PAO,PP,Lo}$	g P.m^{-3}															X						
Non-releasable stored polyP	$X_{PAO,PP,Hi}$	g P.m^{-3}															X						
Other																							
Alkalinity ($CaCO_3$)	S_{Alk}	mol $CaCO_3$.m^{-3}	X			X			X			X									X		
Total suspended solids	X_{TSS}	g TSS.m^{-3}				X			X			X									X		

Table
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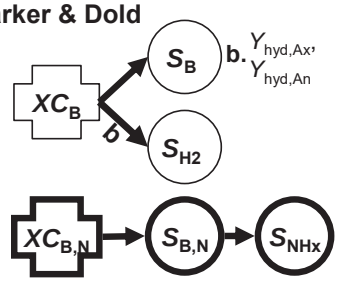
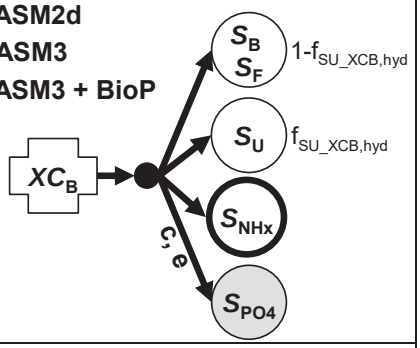
Table 5. Hydrolysis of particulate substrate

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

Organics:

a: $$q_{XCB\_SB,hyd} \cdot M\left(\frac{XC_B}{X_{OHO}}\right) \cdot \left\langle \begin{matrix} M(S_{O2}) \\ \eta_{qhyd,Ax} \cdot M(S_{NOx}) \cdot IM(S_{O2}) \end{matrix} \right\rangle \cdot X_{OHO}$$

b, c: $$q_{XCB\_SB,hyd} \cdot M\left(\frac{XC_B}{X_{OHO}}\right) \cdot \left\langle \begin{matrix} M(S_{O2}) \\ \eta_{qhyd,Ax} \cdot M(S_{NOx}) \cdot IM(S_{O2}) \\ \eta_{qhyd,An} \cdot IM(S_{NOx}) \cdot IM(S_{O2}) \end{matrix} \right\rangle \cdot X_{OHO}$$

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

Particulate nitrogen hydrolysis:

a, b: $$q_{XCB\_SB,hyd} \cdot M\left(\frac{XC_{B,N}}{X_{OHO}}\right) \cdot \left\langle \begin{matrix} M(S_{O2}) \\ \eta_{qhyd,Ax} \cdot M(S_{NOx}) \cdot IM(S_{O2}) \\ \eta_{qhyd,An} \cdot IM(S_{NOx}) \cdot IM(S_{O2}) \end{matrix} \right\rangle \cdot X_{OHO}$$

Ammonification:

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

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

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

Table
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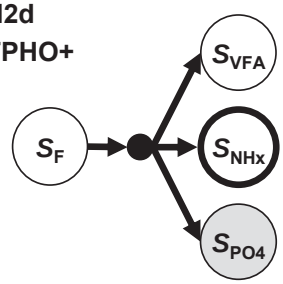
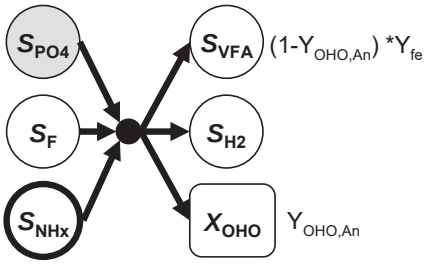
Table 6. Fermentation process

Concept 1: Transformation	**Concept 2:** Anaerobic growth process
An **c. ASM2d** An **g. UCTPHO+**  **c:** $q_{SF\_VFA,Max} \cdot M(S_F) \cdot IM(S_{O2}) \cdot IM(S_{NOx}) \cdot X_{OHO}$ **g:** $q_{SF\_VFA,Max} \cdot S_F \cdot IM(S_{O2}) \cdot IM(S_{NOx}) \cdot X_{OHO}$	An **b. Barker & Dold**  $q_{SF\_VFA,Max} \cdot M(S_F) \cdot M(S_{NHx}) \cdot M(S_{PO4}) \cdot IM(S_{O2}) \cdot IM(S_{NOx}) \cdot X_{OHO}$

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

Table

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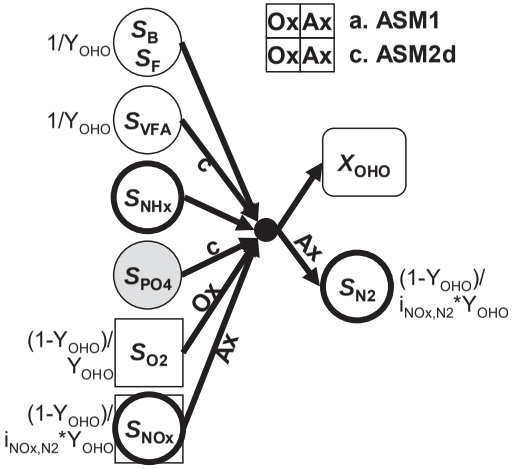
Table 7. OHO growth process concepts

Concept 1: direct growth

Concept 1a: S_{NHx} as only nitrogen source

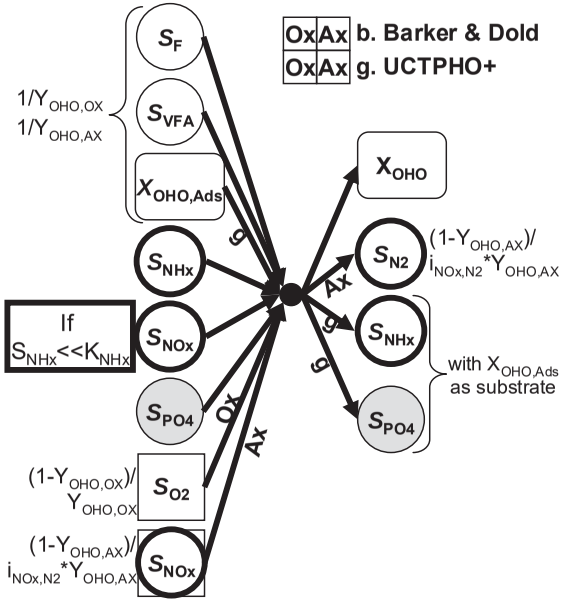


Concept 1b: S_{NHx} or S_{NOx} as nitrogen source

Adsorption

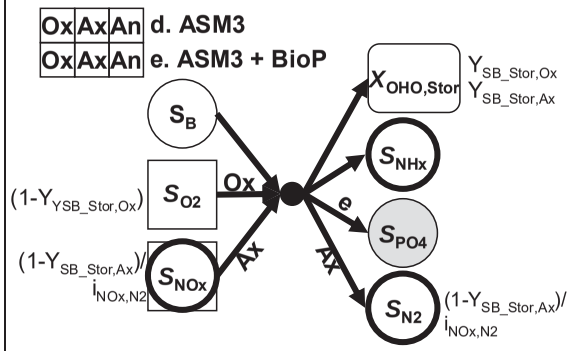
Ox Ax An g. UCTPHO+ XC_B → $X_{OHO,Ads}$

Growth

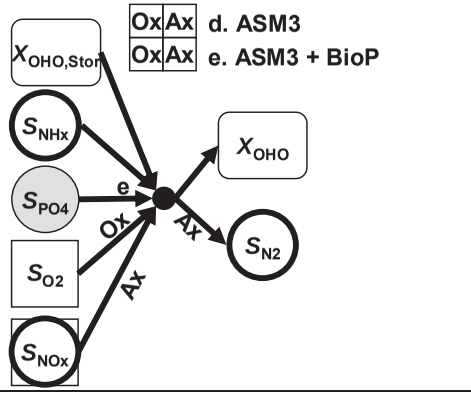


Concept 2: storage - growth

Storage



Growth



Adsorption: $q_{XCB\_Ads} \cdot XC_B \left(f_{Ads\_OHO,Max} - \frac{X_{Ads}}{X_{OHO}} \right) \cdot X_{OHO}$

Growth: With S_{Sub} the considered substrate (S_B, S_F or S_{VFA})

$$\left\langle \begin{matrix} \mu_{OHO,Max} \left[M(S_{Sub}) \frac{S_{Sub}}{\sum_i S_{Sub,i}} \right] \\ \mu_{Ads\_OHO,Max} \left[M\left(\frac{X_{Ads}}{X_{OHO}}\right) \frac{S_{Sub}}{\sum_i S_{Sub,i}} \right] \end{matrix} \right\rangle \cdot \left\langle \frac{M(S_{O2x})}{\eta_{\mu OHO,Ax} M(S_{NOx}) \cdot IM(S_{O2x})} \right\rangle \cdot \langle M(S_{PO4}) \rangle \cdot \left\langle \frac{M(S_{NHx})}{M(S_{NOx}) \cdot IM(S_{NHx})} \right\rangle \cdot X_{OHO}$$

Storage:

$$q_{SB\_Stor} \cdot M(S_B) \cdot \left\langle \frac{M(S_{O2x})}{\eta_{\mu OHO,Ax} M(S_{NOx}) \cdot IM(S_{O2x})} \right\rangle \cdot X_{OHO}$$

Growth:

$$\mu_{OHO,Max} \cdot M\left(\frac{X_{OHO,Stor}}{X_{OHO}} \right) \cdot \left\langle \frac{M(S_{O2x})}{\eta_{\mu OHO,Ax} M(S_{NOx}) \cdot IM(S_{O2x})} \right\rangle \cdot \langle M(S_{PO4}) \rangle \cdot M(S_{NHx}) \cdot X_{OHO}$$

Table
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Table 8. Stoichiometry for OHO growth

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

Table
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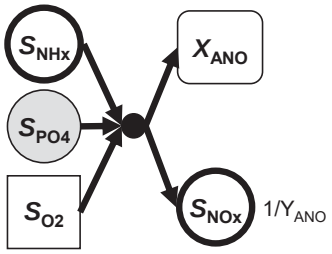
Table 9. ANO growth process

Ox	a. ASM1	
Ox	b. Barker & Dold	
Ox	c. ASM2d	
Ox	d. ASM3	
Ox	e. ASM3 + BioP	
Ox	f. ASM2d + TUD	
Ox	g. UCTPHO+	

$$\mu_{ANO,Max} \cdot M(S_{NHx}) \cdot \langle M(S_{PO4}) \rangle \cdot M(S_{O2}) \cdot X_{ANO}$$

Table
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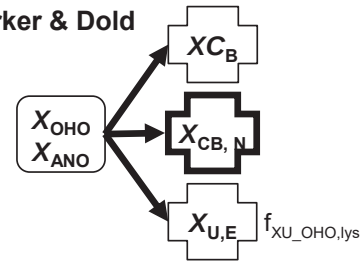
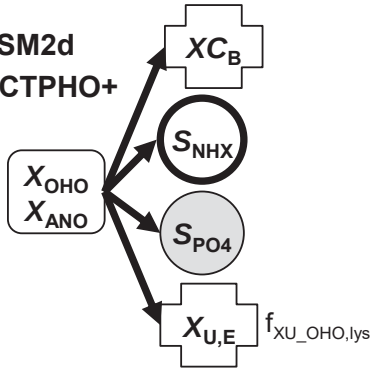
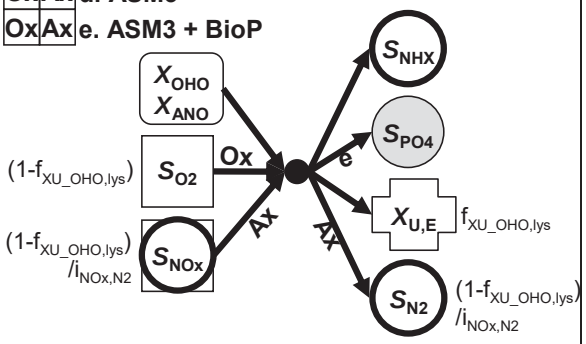
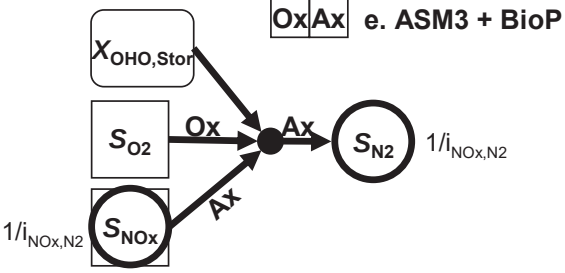
Table 10. OHO and ANO decay process concepts

<table>
<tr>
<th>Concept 1: Death-Regeneration concept</th>
<th>Concept 2: Endogenous respiration concept</th>
</tr>
<tr>
<td>
Concept 1a: nutrients considered separately from substrate
Ox Ax An a. ASM1
Ox Ax An b. Barker & Dold


Concept 1b: nutrients linked to substrate
Ox Ax An c. ASM2d
Ox Ax An g. UCTPHO+


Heterotrophs:
$b_{OHO} \cdot X_{OHO}$
Autotrophs:
$b_{ANO} \cdot X_{ANO}$
</td>
<td>
Ox Ax d. ASM3
Ox Ax e. ASM3 + BioP


Storage lysis (OHOs only)
Ox Ax d. ASM3
Ox Ax e. ASM3 + BioP


Heterotrophs:
$\left\langle \begin{matrix} m_{OHO,Ox} \cdot M(S_{O2}) \\ m_{OHO,Ax} \cdot M(S_{NOx}) \cdot IM(S_{O2}) \end{matrix} \right\rangle \cdot X_{OHO}$
Storage lysis (OHOs only)
$\left\langle \begin{matrix} m_{Stor,Ox} \cdot M(S_{O2}) \\ m_{Stor,Ax} \cdot M(S_{NOx}) \cdot IM(S_{O2}) \end{matrix} \right\rangle \cdot X_{OHO,Stor}$
Autotrophs:
$\left\langle \begin{matrix} m_{ANO,Ox} \cdot M(S_{O2}) \\ m_{ANO,Ax} \cdot M(S_{NOx}) \cdot IM(S_{O2}) \end{matrix} \right\rangle \cdot X_{ANO}$
</td>
</tr>
</table>

N.B.: ASM2d+TUD is identical to ASM2d.
The symbol ‹ › is used to indicate optional or alternative terms, one or none of the lines should be chosen.

Table

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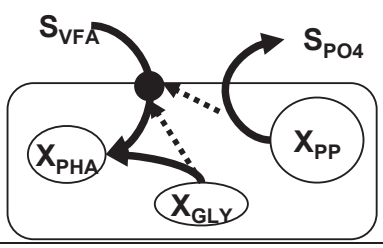
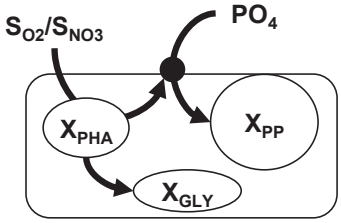
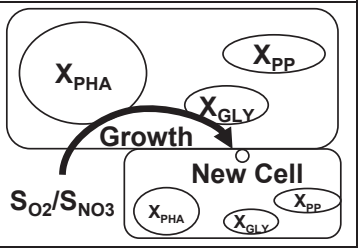
Table 11. Simplified representation of phosphorus accumulating organisms growth

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

Table
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Table 12. PHA storage process concept

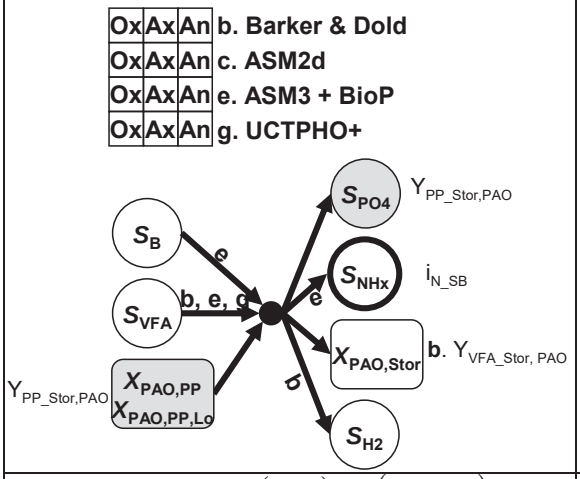
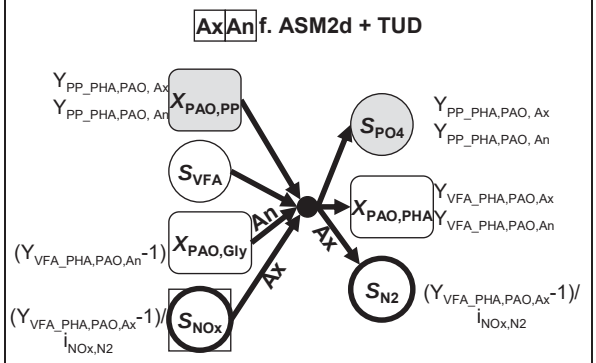
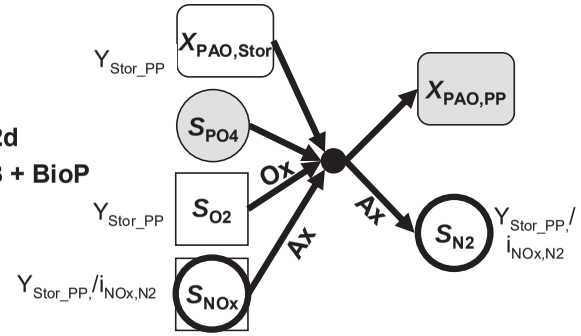
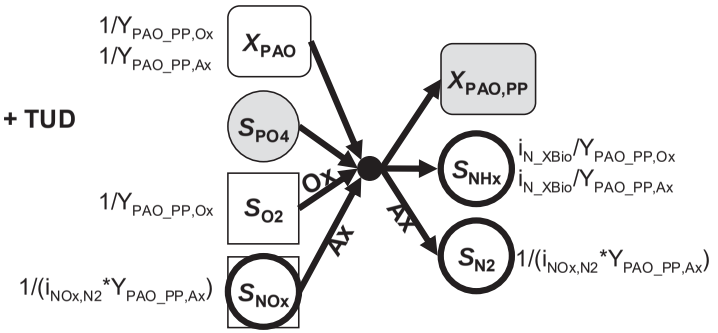
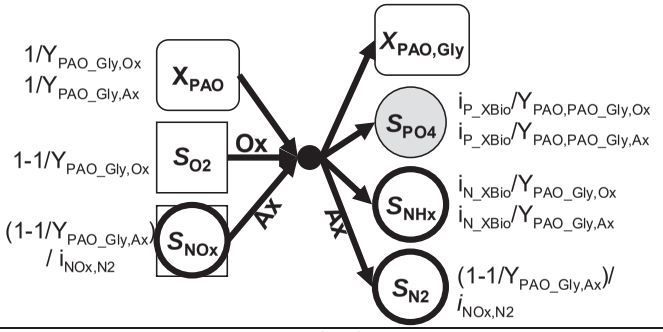
Concept 1: Energy from polyP, reducing power neglected	**Concept 2:** Energy from polyP, reducing power from glycogen
	
b, g: $q_{PAO,VFA\_Stor} \cdot M(S_{VFA}) \cdot M(X_{PAO,PP}) \cdot X_{PAO}$ **c, e**: $q_{PAO,VFA\_Stor} \cdot \left\langle \begin{matrix} M(S_{VFA}) \\ M(S_B) \end{matrix} \right\rangle \cdot M(X_{PAO,PP} / X_{PAO}) \cdot X_{PAO}$	$\left\langle \begin{matrix} q_{PAO,VFA\_PHA,An} \cdot IM(S_{O2}) \cdot IM(S_{NOx}) \cdot M(X_{PAO,Gly}) \\ q_{PAO,VFA\_PHA,Ax} \cdot IM(S_{O2}) \cdot M(S_{NOx}) \end{matrix} \right\rangle$ $\cdot M(S_{VFA}) \cdot M(X_{PAO,PP}) \cdot X_{PAO}$

Table
Click here to download Table: Table13.doc

Table 13. PolyP storage process concept

Concept 1: Uncoupled processes	**Concept 2:** Coupled processes (metabolic model)
Ox Ax **c. ASM2d** Ox Ax **e. ASM3 + BioP** 	**PP storage:** Ox Ax **f. ASM2d + TUD**  **Glycogen storage:** Ox Ax **f. ASM2d + TUD** 
$q_{PAO,PO4\_PP} \cdot \left\langle \frac{M(S_{O2})}{\eta_{qPAO,Ax} \cdot IM(S_{O2}) \cdot M(S_{NOx})} \right\rangle \cdot M(S_{PO4}) \cdot M(X_{PAO,PHA}) \cdot M(f_{PP\_PAO,Max} - X_{PAO,PP} / X_{PAO}) \cdot X_{PAO}$	**PP storage:** $q_{PAO,PO4\_PP} \cdot \left\langle \frac{M(S_{O2})}{\eta_{qPAO,Ax} \cdot IM(S_{O2}) \cdot M(S_{NOx})} \right\rangle \cdot [X_{PAO} / X_{PAO,PP}] \cdot M(S_{PO4}) \cdot M(X_{PAO,PHA}) \cdot M(f_{PP\_PAO,Max} - X_{PAO,PP} / X_{PAO}) \cdot X_{PAO}$ **Glycogen storage:** $q_{Gly} \cdot \left\langle \frac{M(S_{O2})}{\eta_{qPAO,Ax} \cdot IM(S_{O2}) \cdot M(S_{NOx})} \right\rangle \cdot [X_{PAO,PHA} / X_{PAO,Gly}] \cdot M(X_{PAO,PHA}) \cdot M(f_{Gly\_PAO,Max} - X_{PAO,Gly} / X_{PAO}) \cdot X_{PAO}$

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

Table

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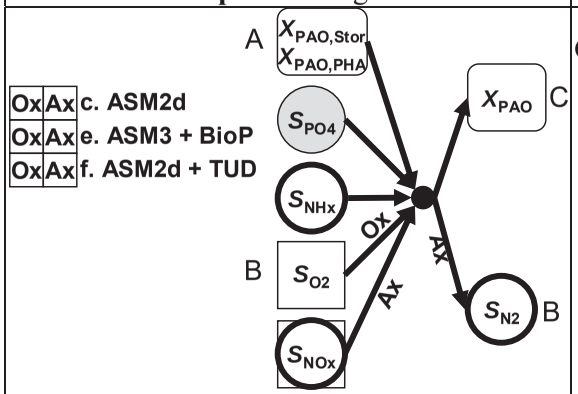
Table 14. PAO growth

Concept 1: PAO growth



Ox	Ax	
Ox	Ax	c. ASM2d
Ox	Ax	e. ASM3 + BioP
Ox	Ax	f. ASM2d + TUD

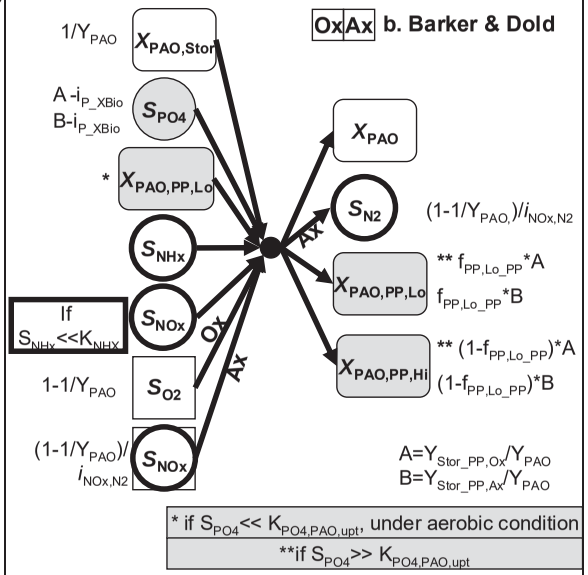
	A	B	C
c.	$1/Y_{Stor\_PAO}$	$1-1/Y_{Stor\_PAO}$	1
e.	$1/Y_{Stor\_PAO,Ox}$ $1/Y_{Stor\_PAO,Ax}$	$1-1/Y_{Stor\_PAO,Ox}$ $1-1/(i_{NOx,N2}*Y_{Stor\_PAO,Ax})$	1
f.	1	$1-1/Y_{Stor\_PAO,Ox}$ $1-1/(i_{NOx,N2}*Y_{Stor\_PAO,Ax})$	$1/Y_{Stor\_PAO,Ox}$ $1/Y_{Stor\_PAO,Ax}$

c, e: $\mu_{PAO,Max}\cdot\left\langle\frac{M(S_{O2})}{\eta_{\mu PAO}\cdot IM(S_{O2})\cdot M(S_{NOx})}\right\rangle\cdot M\left(\frac{X_{PAO,Stor}}{X_{PAO}}\right)\cdot M(S_{NHx})\cdot M(S_{PO4})\cdot X_{PAO}$

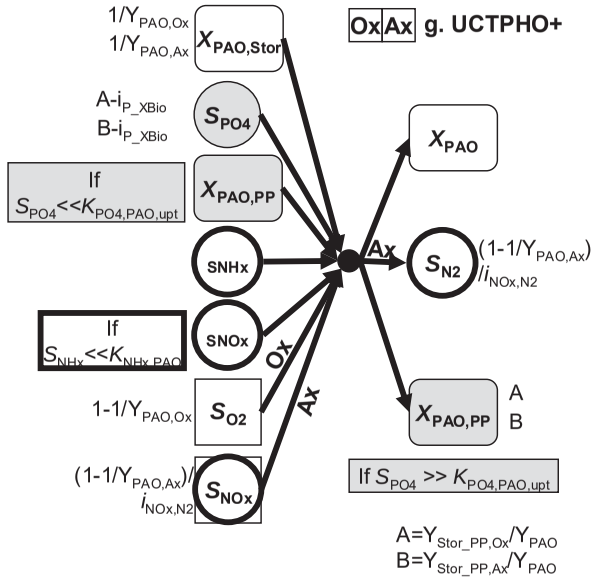
d: $q_{PHA\_PAO}\cdot\left\langle\frac{M(S_{O2})}{\eta_{\mu PAO}\cdot IM(S_{O2})\cdot M(S_{NOx})}\right\rangle\cdot M\left(\frac{X_{PAO,PHA}}{X_{PAO}}\right)\cdot M(S_{NHx})\cdot M(S_{PO4})\cdot X_{PAO}$

Concept 2: Simultaneous growth and polyP storage

Concept 2a: Two polyP storage pools



Concept 2b: Single polyP storage pools



$$\left\langle\frac{\mu_{PAO,Max}}{\mu_{PAO,Max,Plim}}\right\rangle\cdot\left\langle\frac{M(S_{O2})}{\eta_{\mu PAO}\cdot IM(S_{O2})\cdot M(S_{NOx})}\right\rangle\cdot M(X_{PAO,Stor}/X_{PAO})\cdot\left\langle\frac{M(S_{NHx})}{IM(S_{NHx})\cdot M(S_{NOx})}\right\rangle\cdot\left\langle\frac{M(S_{PO4})}{IM(S_{PO4})\cdot M(X_{PAO,PP})}\right\rangle\cdot X_{PAO}$$

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

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

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

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

Table
Click here to download Table: Table17.doc

Table 17: PAO decay process concepts

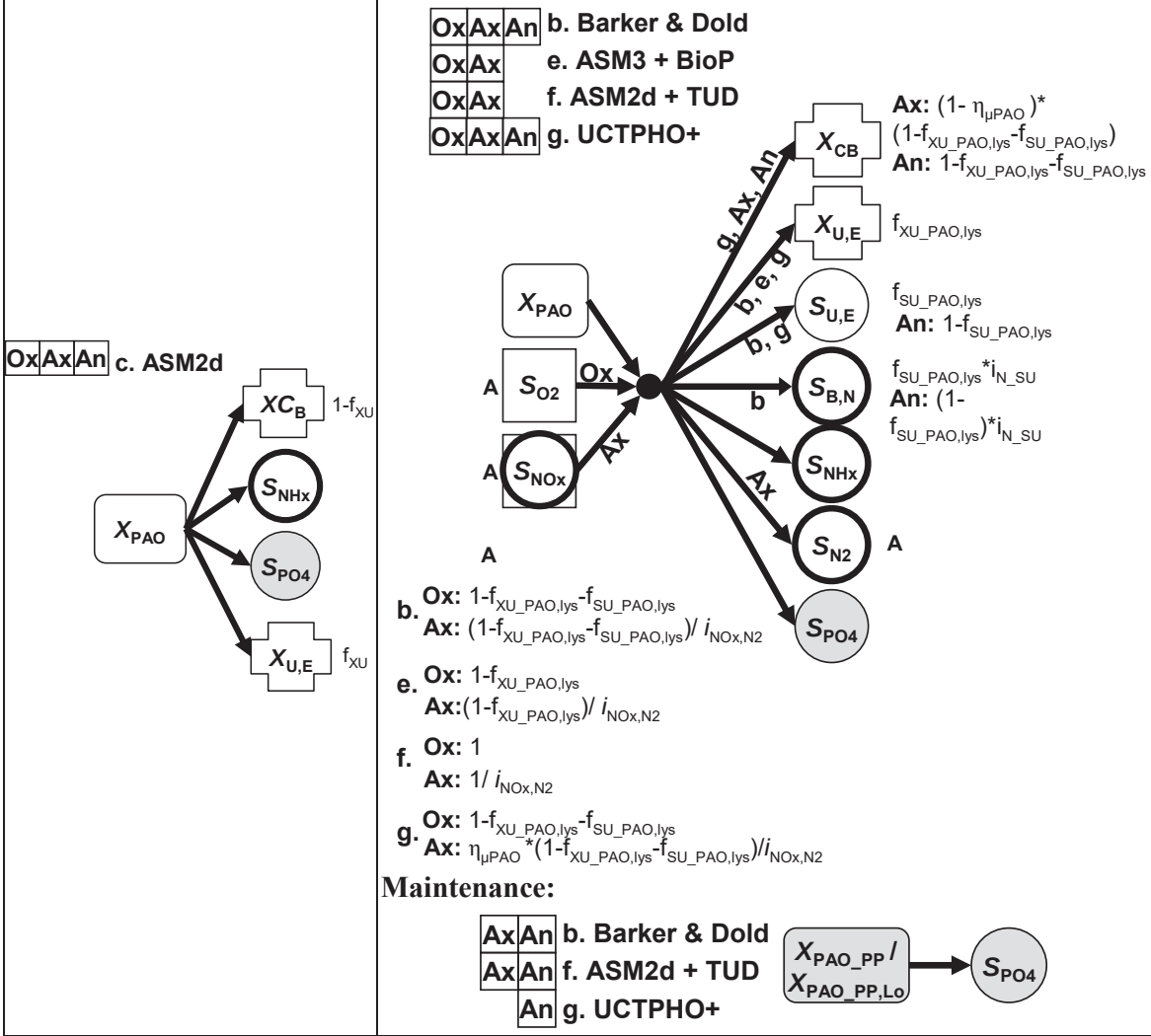
<table>
<tr><th>**Concept 1:** Death-regeneration concept</th><th colspan="2">**Concept 2:** Endogenous respiration</th></tr>
<tr><td> 
</td><td></td></tr>
<tr><td>$b_{PAO} \cdot X_{PAO}$</td><td>**Decay:**</td><td>**b, g:** $m_{PAO} \cdot \left\langle \begin{matrix} M(S_{O2}) \\ IM(S_{O2}) \cdot M(S_{NOx}) \\ IM(S_{O2}) \cdot IM(S_{NOx}) \end{matrix} \right\rangle \cdot X_{PAO}$
e: $m_{PAO} \cdot \left\langle \begin{matrix} M(S_{O2}) \\ \eta_{mPAO} \cdot IM(S_{O2}) \cdot M(S_{NOx}) \end{matrix} \right\rangle \cdot X_{PAO}$
f: $\left\langle \begin{matrix} m_{PAO,Ox} \cdot M(S_{O2}) \\ m_{PAO,Ax} \cdot IM(S_{O2}) \cdot M(S_{NOx}) \end{matrix} \right\rangle \cdot X_{PAO}$</td></tr>
<tr><td></td><td>**Maintenance:**</td><td>**b:** $b_{PP\_PO4} \cdot IM(S_{O2}) \cdot M(X_{PAO,PP,Lo}) \cdot X_{PAO}$
f: $m_{PAO,An} \cdot IM(S_{O2}) \cdot IM(S_{NOx}) \cdot M(X_{PAO,PP}) \cdot X_{PAO}$
g: $b_{PP\_PO4} \cdot \left\langle \begin{matrix} (1-\eta_{\mu PAO}) \cdot IM(S_{O2}) \cdot M(S_{NOx}) \\ IM(S_{O2}) \cdot IM(S_{NOx}) \end{matrix} \right\rangle \cdot M(X_{PAO,PP}) \cdot X_{PAO}$</td></tr>
</table>

Table
Click here to download Table: Table18.doc

Table 18: PAO storage pools release/consumption during lysis

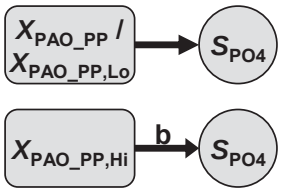
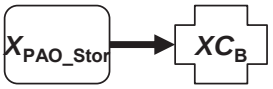
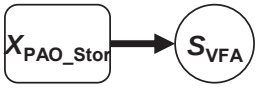
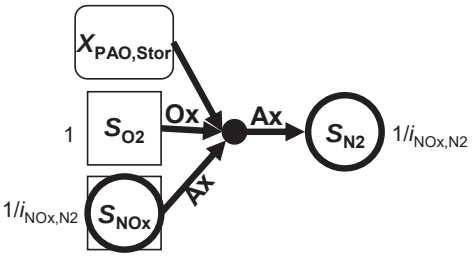
Concept 1: Stored compounds are released	**Concept 2:** Stored compounds are consumed
PolyP lysis: Ox Ax An **b. Barker & Dold** Ox Ax An **c. ASM2d** Ox Ax **e. ASM3 + BioP** Ox Ax An **g. UCTPHO+**  **PHA lysis:** Ox Ax An **g. UCTPHO+**  Ox Ax An **b. Barker & Dold** Ox Ax An **c. ASM2d** 	Ox Ax An **e. ASM3 + BioP** 
PolyP lysis: **b, g:** $m_{PAO} \cdot \left\langle \begin{matrix} M(S_{O2}) \\ IM(S_{O2}) \cdot M(S_{NOx}) \\ IM(S_{O2}) \cdot IM(S_{NOx}) \end{matrix} \right\rangle \cdot \left\langle \begin{matrix} X_{PAO,PP,Lo} / X_{PAO} \\ X_{PAO,PP,Hi} / X_{PAO} \end{matrix} \right\rangle \cdot X_{PAO}$ **c:** $b_{PP\_PAO} \cdot X_{PAO,PP}$ **e:** $b_{PP\_PAO} \cdot \left\langle \begin{matrix} M(S_{O2}) \\ \eta_{bPP\_PAO} \cdot IM(S_{O2}) \cdot M(S_{NOx}) \end{matrix} \right\rangle \cdot X_{PAO,PP}$ **PHA lysis:** **b:** $m_{PAO} \cdot \left\langle \begin{matrix} M(S_{O2}) \\ IM(S_{O2}) \cdot M(S_{NOx}) \\ IM(S_{O2}) \cdot IM(S_{NOx}) \end{matrix} \right\rangle \cdot \frac{X_{PAO,Stor}}{X_{PAO}} \cdot X_{PAO}$ **c:** $b_{Stor\_PAO} \cdot X_{PAO,Stor}$ **g:** $m_{PAO} \cdot \left\langle \begin{matrix} M(S_{O2}) \\ IM(S_{O2}) \cdot M(S_{NOx}) \\ IM(S_{O2}) \cdot IM(S_{NOx}) \end{matrix} \right\rangle \cdot M(S_{NHx}) \cdot M(S_{PO4}) \cdot \frac{X_{PAO,Stor}}{X_{PAO}} \cdot X_{PAO}$	$m_{PAO,Stor} \cdot \left\langle \begin{matrix} M(S_{O2}) \\ \eta_{mPAO,Stor} \cdot IM(S_{O2}) \cdot M(S_{NOx}) \end{matrix} \right\rangle \cdot X_{PAO,Stor}$

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

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

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

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

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

	State variables / Substrate	Nutrients	Aerobic conditions	Anoxic conditions	Anaerobic conditions	Simplified mechanisms
Hydrolysis (3.2.1.3)	XC_B as only particulate fraction	component-based models (**ASM1** and **Barker & Dold**): ammonification			**ASM1** and **UCTPHO+:** anaerobic hydrolysis not considered	chemical dissolution, mass transport, storage (except **ASM3**, **ASM3 +BioP**)
Fermentation (3.2.2.3)					**ASM2d** and **UCTPHO+:** anaerobic biomass production neglected	**ASM3+BioP:** fermentation neglected
OHO Growth (3.2.3.3)	See Table 8. - **ASM3**: poor predictions for non constant growth and storage rates (SRT<5d, long feast/famine cycles…)	- **ASM1** and **ASM3:** P not limiting - **UCTPHO+**, **Barker & Dold:** NO_X as N source	**ASM3:** direct growth on S_B not considered	- **ASM1**, **ASM2d:** constant yields - **ASM3:** direct growth on S_B not considered		- one-step denitrification - inhibitory effects
ANO Growth (3.2.4.3)						one-step nitrification
OHO & ANO decay (3.2.5.3)	death-regeneration: substrate available only for OHOs				endogenous respiration concept (**ASM3**, **ASM3+BioP**): anaerobic decay not considered	dormancy, maintenance, predation
PHA storage (3.3.1.3)	glycogen neglected (except **ASM2d+TUD**) **ASM3+BioP:** fermentation neglected					- competition with GAOs - effect of pH on acetate uptake (except **ASM2d+TUD**)
PolyP storage (3.3.2.3)	**Barker & Dold:** two polyP storage pools			**ASM2d** and **ASM3+BioP:** constant yields		biologically induced phosphate precipitation
PAO growth (3.3.3.3)	**ASM2d, ASM3+BioP**: growth and polyP storage not linked	**UCTPHO+** and **Barker & Dold** only: NO_X as N source and polyP as P source	direct growth on S_{VFA} and S_F not considered	**ASM2d** and **Barker & Dold:** constant yield		
PAO decay (3.3.4.3)			aerobic maintenace not considered	**Barker & Dold, UCTPHO+**: constant rate	**- ASM3+BioP:** anaerobic decay and anaerobic maintenance not modelled **- Barker & Dold, UCTPHO+**: constant rate	PHA and glycogen utilisation for maintenance are not considered
PAO storage pool lysis (3.3.4.3)	**UCTPHO+:** PHA released in the form of XC_B		**ASM3+BioP**: electron acceptor consumed	**ASM3+BioP**: electron acceptor consumed		**ASM2d+TUD:** Storage pool lysis not modelled

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

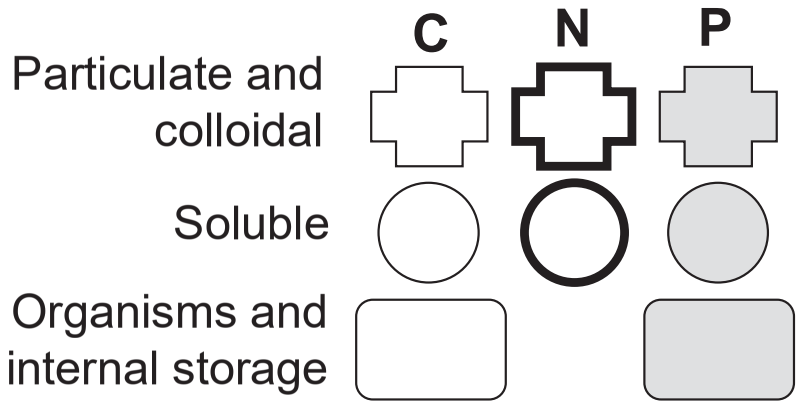
Needs in research	Modelling process affected	Current modelling concept or solution	Modelling problem	Research needs in practice and in understanding
OHO and ANO processes				
Predation (e.g. protozoa activity, bacteriophage)	hydrolysis, biomass decay (concern PAO decay also)	not included in ASM type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

Figure

Models

a. ASM1
b. Barker & Dold
c. ASM2d
d. ASM3
e. ASM3 + BioP
f. ASM2d + TUD
g. UCTPHO+

State Variables



Electron acceptor conditions

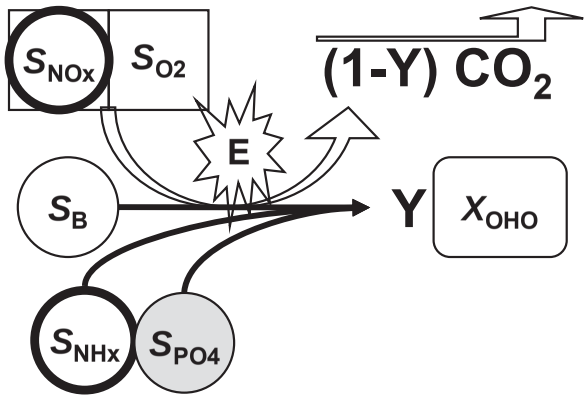
Ox Aerobic
Ax Anoxic
An Anaerobic

Electron acceptor

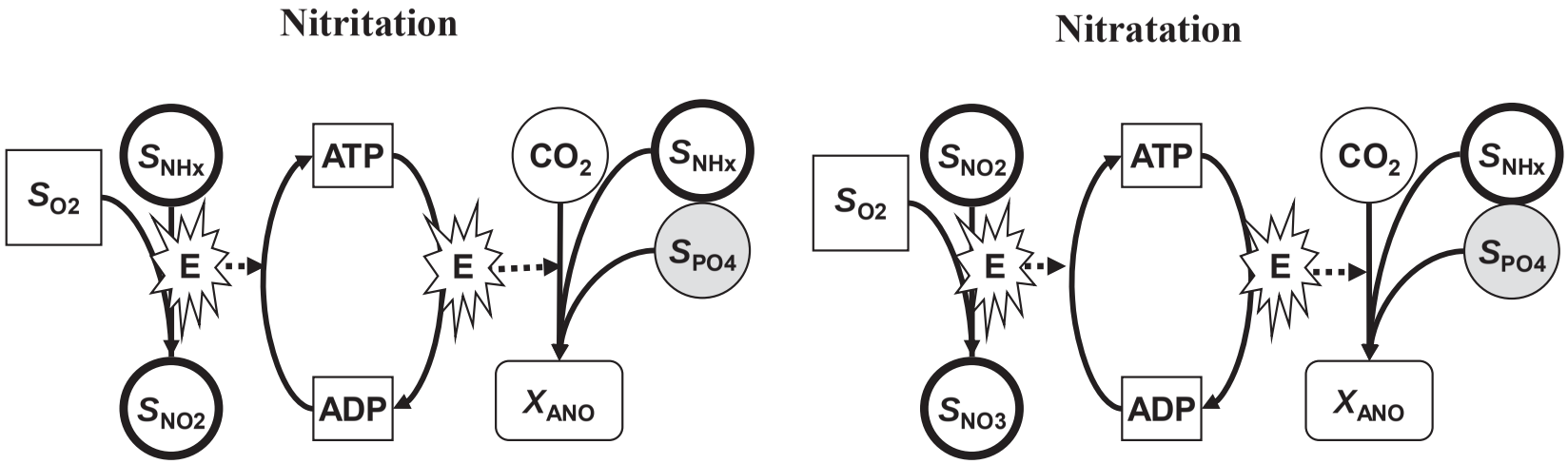
S_{O2} Oxygen

S_{NOx} Nitrate/nitrite

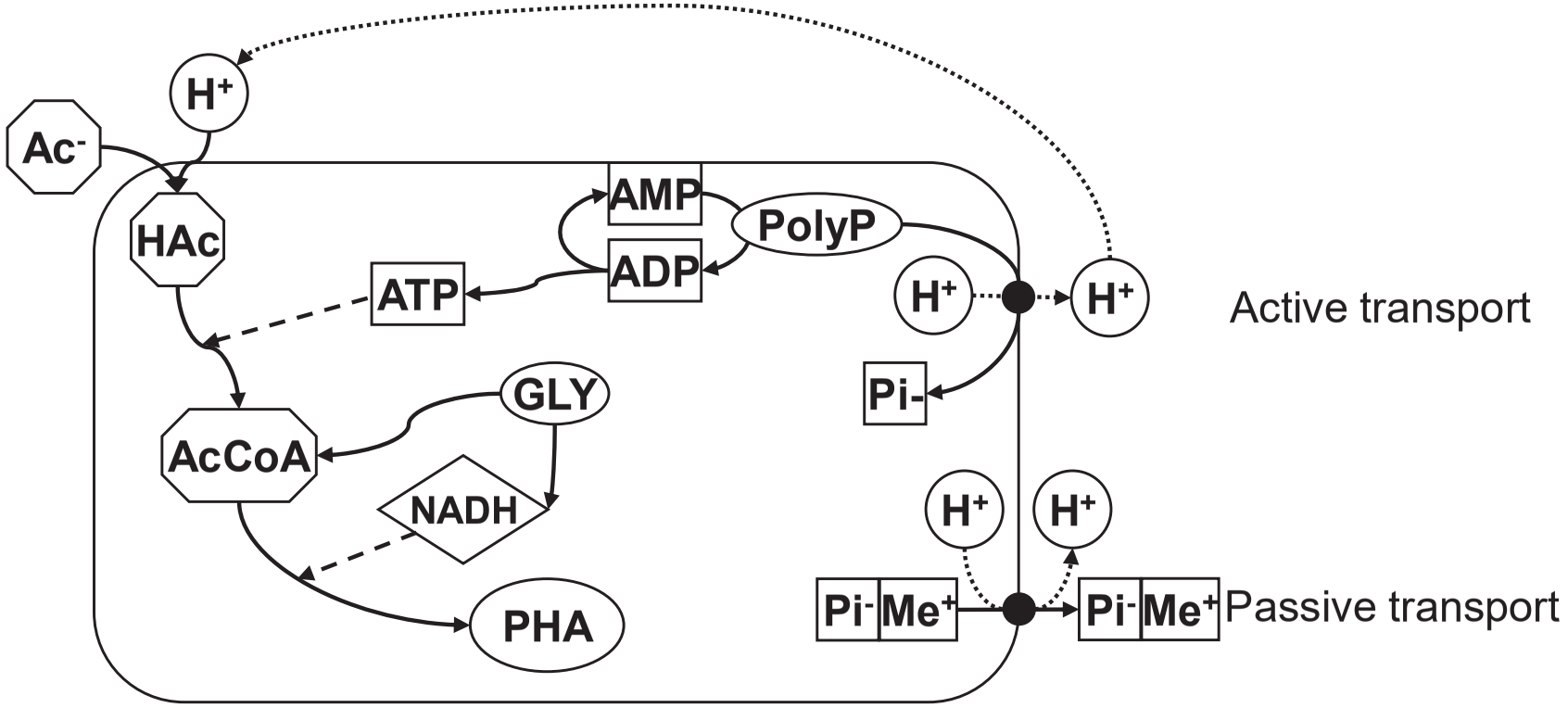
Figure



Figure


Nitritation
S_{O2}
S_{NHx}
E
S_{NO2}
ATP
ADP
E
CO_2
S_{NHx}
S_{PO4}
X_{ANO}
Nitratation
S_{O2}
S_{NO2}
E
S_{NO3}
ATP
ADP
E
CO_2
S_{NHx}
S_{PO4}
X_{ANO}

Figure


H+
Ac-
HAc
AMP
PolyP
ADP
ATP
H+
H+
Active transport
Pi-
GLY
AcCoA
NADH
H+
H+
PHA
Pi-Me+
Pi-Me+
Passive transport