CODEN:LUTEDX/(TEIE-7281)/1-36/(2021)

Industrial Electrical Engineering and Automation

Wastewater treatment process models for enhanced biological phosphorus removal and VFA-production
– A literature review

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Åsa, Sweden, May, 2021

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1 Introduction

The purpose of this document is to describe the different ways of describing *enhanced biological phosphorus removal* (EBPR; sometimes referred to as biological enhanced phosphorus removal/biological excess phosphorus removal, BEPR, or simply bio-P) in the form of mathematical models. Focus is on describing the mechanisms of EBPR and the production of volatile fatty acids (VFA) through primary sludge hydrolysis and fermentation, as well as limitations of the different models. The aim is to provide an overview of both well-established models as well as newly developed models, but not to cover all possible models. For a more detailed description of the different models, the reader is referred to the original publications.

2 Mechanisms for enhanced biological phosphorus removal – brief history and recent research

2.1 Conventional EBPR

Microorganisms need access to nutrients such as nitrogen (N) and phosphorus (P) to be able to grow, as these are an essential part of the cell structure. Thus, in growth processes occurring in wastewater treatment systems, e.g., for ordinary heterotrophic organisms (OHO) and autotrophic nitrifying organisms (ANO), part of the new cell mass consists of nitrogen and phosphorus incorporated from the bulk liquid into the cells. For these types of organisms, the phosphorus content is usually around 0.02 g $P.g\ VSS^{-1}$. EBPR refers to the process where certain types of heterotrophic organisms, referred to as polyphosphate accumulating organisms (PAO), can store large amounts of phosphorus in the cells as storage compounds (in the form of polyphosphate, PP). This results in increased capacity for uptake of P, reaching 0.06 - 0.15 g $P.g\ VSS^{-1}$ (Wentzel et al., 2008).

The EBPR process is accomplished by cyclic variation of the environmental conditions which the biomass is subjected to. The first stage is anaerobic (absence of oxygen and nitrite/nitrate), where PAO take up readily degradable substrate in the form of short chain volatile fatty acids (VFA), such as acetate and propionate, and convert it to storage compounds in the form of poly-β-hydroxyalkanoates (PHA). The most common forms of PHA are poly-β-hydroxybutyrate (PHB, formed from acetate) and polyhydroxyvalerate (PHV, formed from acetate and propionate) (Wentzel et al., 2008). To obtain energy for this conversion, other energy rich storage compounds in the cells (glycogen and polyphosphate) are degraded, simultaneously releasing orthophosphate ($H_3PO_4/H_2PO_4^-/HPO_4^{2-}/PO_4^{3-}$) from the cells into the surrounding water. The growth rate of PAO is slower than for OHO, but since OHO cannot utilize VFA for growth in the absence of oxygen or nitrate/nitrite, PAO has a competitive advantage under anaerobic conditions. Still, OHO can utilize readily biodegradable substrate in the form of more complex molecules than VFA to produce VFA through the process of fermentation under anaerobic conditions, thus producing substrate for PAO. The fermentation process is slower than the uptake of VFA and is thus the rate limiting process (Wentzel et al., 2008).

After the anaerobic stage, biomass is subjected to either anoxic conditions, aerobic conditions, or anoxic followed by aerobic conditions. With the access to O_2 or NO_3^-, the PAO can degrade the stored PHA to use it both as a carbon source and gain energy for biomass growth, as well as restore the glycogen reserves that were previously depleted (Wentzel et al., 2008). Part of the energy gained is also used to take up orthophosphate from the bulk liquid and again store it as polyphosphate. When there is a net growth in the activated sludge system, the PAO biomass removes more phosphate than is released under anaerobic conditions and the excess P is removed with the waste sludge. The changes occurring in the PAO cells under the different environmental conditions are shown in Figure 1, while an example of concentration profiles for relevant pollutants is shown in Figure 2.

Favourable conditions for EBPR include a hydraulic retention time in the anaerobic tank of 0.5 – 1 h and an aerobic SRT of 3 – 40 d (Tchobanoglous et al., 2014). Due to the sequential variation in environmental conditions required for the process to function properly, it is important to avoid recycling of oxygen or nitrate to the anaerobic zones through nitrate recycle or return activated sludge. PAO generally form dense flocs that settle well in the secondary clarifier (Tchobanoglous et al., 2014).

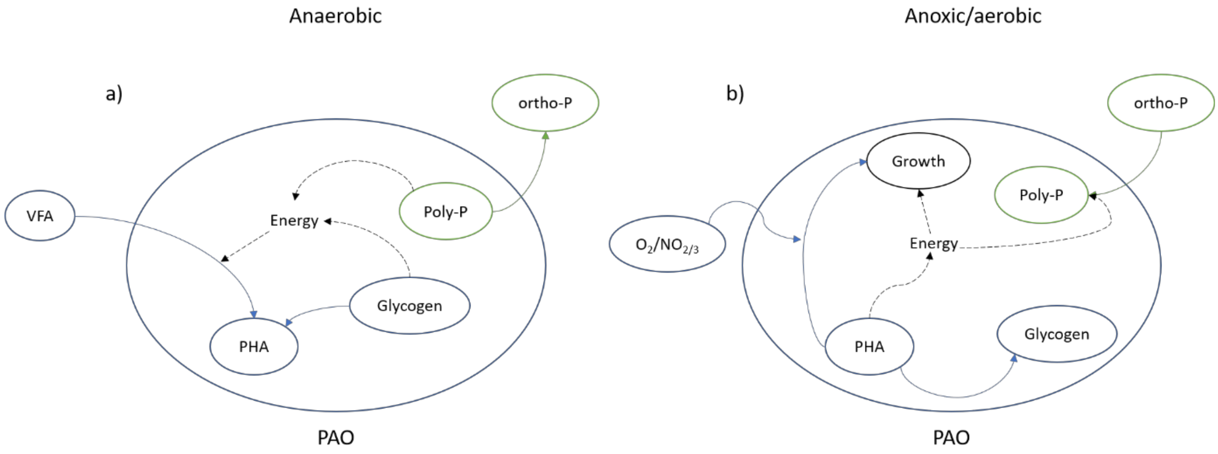

Figure 1. Simplified conceptual model of EBPR reactions for PAO under a) anaerobic and b) anoxic/aerobic conditions. Adapted from Wentzel et al. (2008).

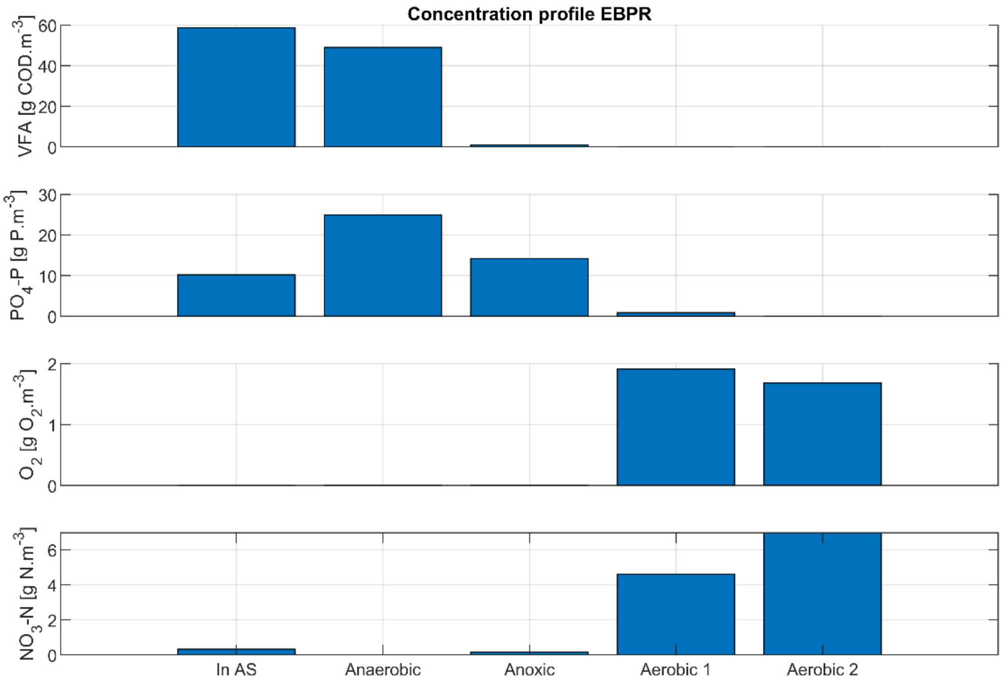

Figure 2. Example of concentration profiles for VFA (measured in COD units), PO_4-P, O_2 and NO_3-N over a EBPR wastewater treatment plant with anaerobic, anoxic and two aerobic zones. Data obtained from simulation using ASM2d (Henze et al., 1999).

One type of organism that, unlike OHO, is a direct competitor to PAO is called *glycogen accumulating organisms*, GAO. GAO can take up VFA under anaerobic conditions and store it internally in the form of PHA, while consuming glycogen that was previously stored under aerobic conditions (as depicted in Figure 3). GAO do thus not store or release orthophosphate but compete with PAO for VFA. Generally, high temperatures (> 20 °C) or low pH (< 7.0) in the anaerobic zone as well as low influent P/COD ratio have been shown to favour GAO over PAO (Wentzel et al., 2008). Other factors that influence the competition have been shown to be the SRT and the division of VFA between acetate and propionate (Tchobanoglous et al., 2014). Onnis-Hayden, Majed et al. (2020) performed extensive tests on the impacts on SRT in the range of 6 – 40 d, where they found that SRT < 10 d was preferable to achieve stable EBPR performance. Longer SRT, such as to allow for nitrification, seemed to also favour higher relative abundance of GAO compared to PAO species. Onnis-Hayden, Srinivasan et al. (2020) identified that in side stream EBPR (S2EBR) processes, such as with return activated sludge (RAS) fermentation (see Section 2.2), the relative abundance of known GAO species in these systems is considerably lower than in conventional systems, while the relative abundance of known PAO species is similar. This suggest that conditions in side stream systems might be unfavourable for GAO species (or that previously unknown GAO proliferate under these conditions), which might be the reason for increased process stability observed for S2EBPR systems (Onnis-Hayden, Srinivasan, et al., 2020).

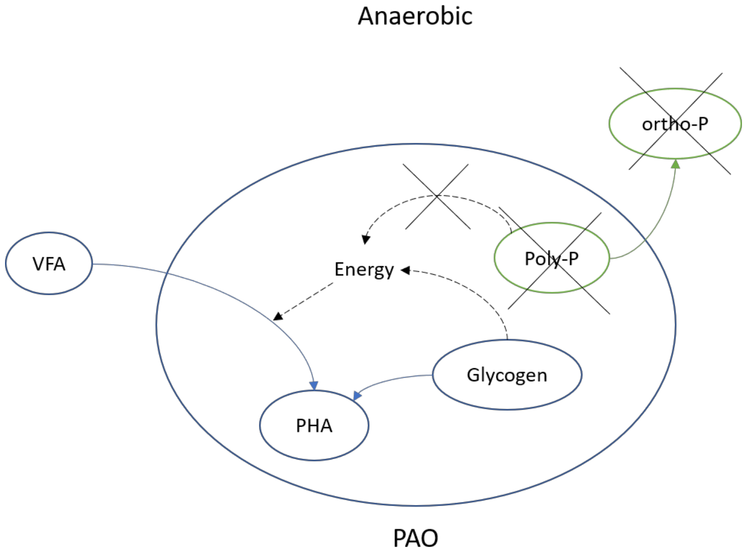

Figure 3. Simplified conceptual model for GAO behaviour under anaerobic conditions, for comparison with PAO behaviour as shown in Figure 1a. Adapted from Wentzel et al. (2008).

The microbial species responsible for EBPR have previously been difficult to identify (Tchobanoglous et al., 2014). Early methods found likely species within the Rhodocyclus group (Bond et al., 1995), later named *Candidatus* Accumulibacter Phosphatis (Hesselmann et al., 1999), as an important PAO due to its abundance at EBPR plants and since it is easily enriched in VFA-fed lab systems (Nielsen et al., 2019). *Tetrasphaera* PAO have later also been found, which are not as well studied and understood as *Ca.* Accumulibacter (Fernando et al., 2019). A majority of *Tetrasphaera* appear not to store PHA (although a few exceptions exist) but instead produce storage products in the form of glycogen and amino acids; additionally, although some can use VFA as substrate, carbohydrates and amino acids seem to be preferred (Kristiansen et al., 2013; Nguyen et al., 2015). This uptake of substrate is performed through fermentation, in which part of the readily biodegradable COD is stored and part of it released as VFA (Dunlap et al., 2016). *Ca.* Accumulibacter are not able to ferment readily biodegradable substrate to VFA and is thus reliant on OHO or *Tetrasphaera* PAO to produce VFA in case the influent concentration is inadequate for EBPR. According to Dunlap et al. (2016), the specific conditions and relative abundance of *Ca*. Accumulibacter vs. *Tetrasphaera* can therefore have a significant impact on model results using the older process models that do not consider the different species and processes (also pointed out by Onnis-Hayden, Srinivasan et al. (2020)), compromising the predictive power of the model. Dunlap et al. (2016) further point to the fact that since *Tetrasphaera* can utilize amino acids in the fermentation process, fermentation of biomass decay products (which mainly contain protein) might favour production of more *Tetrasphaera* than with fermentation of influent carbon.

A recent study of 8 full-scale WWTPs confirmed *Ca*. Accumulibacter and *Tetrasphaera* as the most abundant PAO (measured as contributing the most to the observed EBPR), with *Tetrasphaera* PAO being the dominant PAO type in 6 of the 8 WWTPs (Fernando et al., 2019). Onnis-Hayden, Srivasan, et al. (2020) also found high levels of *Tetrasphaera*, although when the total number of PAO was estimated through staining this number was lower than the sum of *Tetrasphaera* and *Ca*. Accumulibacter (possibly due to overestimation of *Tetrasphaera* in the FISH (fluorescent in-situ hybridization) analysis or because some *Tetrasphaera* are not able to accumulate poly-P). Another recent study also concluded that *Tetrasphaera* is as important as *Ca*. Accumulibacter for EBPR (Nielsen et al., 2019), and that GAO may not be a real problem and cause of failure for full-scale EBPR as no direct examples are found in the literature and stable EBPR has been found even with high abundance of GAO (Stokholm-Bjerregaard et al., 2017). Nielsen et al. (2019) also point out that several examples of successful full-scale EBPR exist for tropical conditions (water temperature 28-32 °C), where EBPR is thought not to be possible due to the competitive advantage of GAO that have been shown for lab studies at high temperatures.

2.2 Side stream EBPR (S2EBPR) processes

Several variations of S2EBPR processes exist. Onnis-Hayden, Srinivasan et al. (2020) describe the following:

- Side stream return activated sludge (RAS) fermentation;
- Side stream RAS fermentation with addition of primary sludge fermentate;
- Side stream mixed liquor suspended solids (MLSS) fermentation;
- Unmixed in-line MLSS fermentation.

Barnhard et al. (2017) proposed that the early identified mechanisms for EBPR led to early design guidelines which resulted in selection of the type of PAO previously identified (such as *Ca*. Accumulibacter). Alternative process configurations, such as RAS fermentation, seem to enable growth of several types of PAO, such as *Tetrasphaera*, which both contributes to EBPR itself but also benefits *Ca*. Accumulibacter by producing VFA in the fermentation process. This creates a synergy which can be the reason for the increased process stability observed in these types of systems (e.g. Onnis-Hayden, Srinivasan et al. (2020)).

Barnhard et al. (2017) also states that the *oxidation-reduction potential* (ORP) in the anaerobic zone can be an important factor as evidence points to that ORP values below -200 mV favours *Tetrasphaera*. They proceed to explain reasons why conventional designs often fail to reach ORP values this low: *i*) too violent mixing in the anaerobic zone, causing oxygen intrusion; *ii*) too large primary effluent flow to the anaerobic zone, when higher flows, such as during wet weather, should instead be diverted to the anoxic zone; *iii*) the anaerobic zone is constructed as a completely mixed reactor, where plug flow designs have been shown to produce lower ORP along the basin; *iv*) too long HRT in the anaerobic zone, which if the ORP is not low enough due to the reasons stated above, further deteriorates the conditions for fermenting PAO such as *Tetrasphaera*; *v*) large RAS flow to the anaerobic zone, which can cause to much nitrate to be recycled as well as decrease the HRT.

Dold & Conidi (2019) argue that the reason for the success of S2EBPR systems compared to conventional systems could be attributed to insufficient design of the anaerobic zone in conventional systems. They point to the fact that guidelines are often lacking for how to design the anaerobic zone depending on the characteristics of the influent COD, and that many plants in North America are built with the anaerobic zone occupying <10% of the volume in the activated sludge basin while often 15-25% is needed. They also point out some disadvantages with RAS fermentation, such as that it mainly utilizes hydrolysis of biomass decay products, while influent COD contains more slowly biodegradable COD with larger potential for VFA production per unit COD. They point to some issues that need to be clarified, mainly: i) there are still inconsistent reports in the literature regarding the impact of *Tetrasphaera* which should be further investigated (for example, the PAO comprise 10-15% of the heterotrophs in an activated sludge system, the impact of *Tetraspaera* fermentation should therefore be low compared to OHO fermentation); ii) issues with quantification procedures for relative abundance of different PAO species have been reported (Rubio-Rincón et al., 2019).

3 Modelling enhanced biological phosphorus removal (EBPR)

Many different models that describe processes for EBPR have been developed and published over the years. In this section, some of the most used models are presented and described along with models published in the last few years with the aim to overcome some of the limitations of the previous models. Since all the models aim to describe EBPR many processes are identical or very similar, but many small and a few large variations can be found. A major division can be made between the models describing the macro-scale conversions observed (such as ASM2d) and models describing the metabolic reactions occurring on a cellular level (such as ASM2d + TUD). Other differences include the description of microbial storage compounds, how many species of PAO and/or GAO that are included and how decay of PAO and the related storage compounds occur. While the above-mentioned models are mechanistic models, i.e., they aim to describe the underlying mechanisms which cause the observed behaviour, some studies where empirical data-driven models have been developed can also be found and one example is described in this review. The models considered in this review, as well as what type of model each is, are listed in Table 1.

Table 1. EBPR models considered and described in this review and the model type each belongs to (describing macro-scale conversions or metabolic reactions).

Model	**Type**	**Reference**
Barker & Dold	Mechanistic, macro	Barker & Dold (1997)
ASM2d	Mechanistic, macro	Henze et al. (1999)
ASM3 + Eawag bio-P module	Mechanistic, macro	Rieger et al. (2001)
ASM2d + TUD	Mechanistic, metabolic	Meijer (2004)
UCTPHO+	Mechanistic, macro	Hu et al. (2007)
ASM2d-N_2O	Mechanistic, macro	Massara et al. (2018)
Sumo EBPR model	Mechanistic, macro	Varga et al. (2018)
META-ASM	Mechanistic, metabolic	Santos et al. (2020)
Lindstrøm Sørensen et al. model	Hybrid mechanistic/data-driven	Lindstrøm Sørensen et al. (2019)

3.1 ASM2d

ASM2 (Gujer et al., 1995) was developed as an extension of the model ASM1 (see Henze et al. (2000)) as part of the work of the International Water Association's (IWA) Task Group on Mathematical Modelling for Design and Operation of Activated Sludge Processes to also describe enhanced biological phosphorus removal. An unresolved question at the time was the ability of PAO to denitrify, which resulted in the decision to exclude denitrifying PAO from the model. In the years following the publication of the model, clear evidence of the capability of PAO to denitrify was demonstrated in several studies, leading to the decision to update ASM2 to include denitrifying PAO. The new updated model was named ASM2d (Henze et al., 1999). ASM2d contains 19 state variables (Table 2) and 21 processes (Table 3), including biological processes for aerobic oxidation of organic material, nitrification, denitrification and EBPR.

Table 2. State variables for ASM2d, names adapted to standardized notation according to Corominas et al. (2010).

State variable name	Unit	Description
S_{O2}	$g\ O_2.m^{-3}$	Dissolved oxygen
S_F	$g\ COD.m^{-3}$	Fermentable, readily biodegradable substrate
S_{VFA}	$g\ COD.m^{-3}$	Fermentation products, such as volatile fatty acids (VFA), assumed to be acetate
S_{NHx}	$g\ N.m^{-3}$	Ammonium and ammonia nitrogen
S_{NOx}	$g\ N.m^{-3}$	Nitrate and nitrite nitrogen
S_{PO4}	$g\ P.m^{-3}$	Ortophosphate phosphorus
S_U	$g\ COD.m^{-3}$	Dissolved unbiodegradable organic material
S_{Alk}	$mole\ HCO_3.m^{-3}$	Alkalinity
S_{N2}	$g\ N.m^{-3}$	Dissolved nitrogen gas
XC_B	$g\ COD.m^{-3}$	Slowly biodegradable organic material (particulate and colloidal)
X_U	$g\ COD.m^{-3}$	Inert particulate organic material
X_{OHO}	$g\ COD.m^{-3}$	Ordinary heterotrophic organisms
X_{PAO}	$g\ COD.m^{-3}$	Polyphosphate accumulating organisms
$X_{PAO,PP}$	$g\ P.m^{-3}$	Polyphosphate stored in PAO
$X_{PAO,Stor}$	$g\ COD.m^{-3}$	Storage products in PAO in the form of PHA
X_{ANO}	$g\ COD.m^{-3}$	Autotrophic nitrifying organisms
X_{TSS}	$g\ TSS.m^{-3}$	Total suspended solids
X_{MeOH}	$g\ MeOH.m^{-3}$	Metal-hydroxides
X_{MeP}	$g\ MeP.m^{-3}$	Metal-phosphates

Dissolved readily biodegradable organic material is divided into two state variables: S_{VFA}, assumed to be short chain volatile fatty acids (VFA) mainly in the form of acetate that can be utilized by both ordinary heterotrophic organisms (OHO) for aerobic oxidation or denitrification, as well as by PAO for storage as PHA; and S_F, fermentable readily biodegradable substrate that can be oxidized directly by OHO under aerobic or anoxic conditions or be fermented by OHO to produce S_{VFA} (which is assumed to occur without biomass growth). It is assumed that no fermentation of S_F is facilitated by PAO. The model does not include glycogen as a storage material in order to reduce model complexity. PAO are assumed to be comprised of one single group of organisms, and the model does not include GAO, which may otherwise compete for S_{VFA} as substrate. Growth of PAO is assumed to occur only on the stored PHA ($X_{PAO,Stor}$) as substrate (under aerobic or anoxic conditions), no direct growth on dissolved substrate such as S_{VFA} is included. S_{PO4} is released from stored polyphosphate ($X_{POA,PP}$) while S_{VFA} is consumed to produce internal storage products in the form of PHA ($X_{PAO,Stor}$), which occurs mainly under anaerobic conditions but can also occur under aerobic/anoxic conditions. During aerobic/anoxic conditions, S_{PO4} is stored in the biomass in the form of $X_{PAO,PP}$ when energy is gained from respiration of $X_{PAO,Stor}$. A maximum allowed polyphosphate storage is defined as $X_{PAO,Stor}/X_{PAO}$ and included as an inhibition term. Storage of S_{PO4} as well as growth under anoxic conditions is assumed slower than under aerobic conditions and is included in the model by a constant anoxic reduction factor. Death, endogenous respiration and maintenance are modelled with decay rate equations for X_{PAO}, $X_{PAO,Stor}$ and $X_{PAO,PP}$ as they all are part of the PAO biomass.

Table 3. Processes included in ASM2d.

Process #	**Process description**	**Process** #	**Process description**
Hydrolysis processes		**Polyphosphate accumulating organisms (cont.)**	
1	Aerobic hydrolysis of XC_B to S_F	12	Anoxic storage of $X_{PAO,PP}$
2	Anoxic hydrolysis of XC_B to S_F	13	Aerobic growth of X_{PAO}
3	Anaerobic hydrolysis of XC_B to S_F	14	Anoxic growth of X_{PAO}
Ordinary heterotrophic organisms		15	Lysis of X_{PAO}
4	Aerobic growth on S_F	16	Lysis of $X_{PAO,PP}$
5	Aerobic growth on S_{VFA}	17	Lysis of $X_{PAO,Stor}$
6	Anoxic growth on S_F		**Autotrophic nitrifying organisms**
7	Anoxic growth on S_{VFA}	18	Aerobic growth of X_{ANO}
8	Fermentation of S_F to S_{VFA}	19	Lysis
9	Lysis	**Simultaneous precipitation**	
Polyphosphate accumulating organisms		20	Precipitation
10	Storage of $X_{PAO,Stor}$	21	Redissolution
11	Aerobic storage of $X_{PAO,PP}$		

Organically bound nitrogen and phosphorus are not included as separate state variables (as was the case for particulate and dissolved biodegradable nitrogen in ASM1), but rather assumed as a fixed content of the COD variables (S_F, S_U, XC_B and X_U). The total nitrogen content thus must be calculated through inclusion of those ratios.

Chemical precipitation of phosphorus by addition of metal salts (mainly for simultaneous precipitation) is included in the model by two simple equations and the state variables X_{MeOH} and X_{MeP}.

3.2 Barker & Dold

The Barker & Dold model (Barker & Dold, 1997) includes processes for aerobic oxidation of organic material, nitrification, denitrification and EBPR. Many similarities with ASM2d are found, with some key aspects and differences from ASM2d described below. The model contains 20 state variables (Table 4) and 36 processes (Table 5).

Table 4. State variables included in the Barker & Dold model, names adapted to standardized notation according to Corominas et al. (2010).

State variable name	Unit	Description
S_{O2}	g O_2.m^{-3}	Dissolved oxygen
S_F	g COD.m^{-3}	Fermentable, readily biodegradable substrate
S_{VFA}	g COD.m^{-3}	Fermentation products, such as volatile fatty acids (VFA), assumed to be acetate
S_{NHx}	g N.m^{-3}	Ammonium and ammonia nitrogen
S_{NOx}	g N.m^{-3}	Nitrate and nitrite nitrogen
S_{PO4}	g P.m^{-3}	Ortophosphate phosphorus
S_U	g COD.m^{-3}	Dissolved unbiodegradable organic material
S_{N2}	g N.m^{-3}	Dissolved nitrogen gas
$S_{B,N}$	g N.m^{-3}	Soluble biodegradable organic nitrogen
$S_{N,U}$	g N.m^{-3}	Soluble inert organic nitrogen
XC_B	g COD.m^{-3}	Slowly biodegradable organic material (particulate and colloidal)
$X_{U,inf}$	g COD.m^{-3}	Inert particulate organic material
$X_{U,E}$	g COD.m^{-3}	Particulate unbiodegradable endogenous products
X_{OHO}	g COD.m^{-3}	Ordinary heterotrophic organisms
X_{PAO}	g COD.m^{-3}	Polyphosphate accumulating organisms
$X_{PAO,PP,Lo}$	g P.m^{-3}	Releasable polyphosphate stored in PAO
$X_{PAO,PP,Hi}$	g P.m^{-3}	Non-releasable polyphosphate stored in PAO
$X_{PAO,Stor}$	g COD.m^{-3}	Storage products in PAO in the form of PHA
$XC_{B,N}$	g N.m^{-3}	Particulate biodegradable organic nitrogen
X_{ANO}	g COD.m^{-3}	Autotrophic nitrifying organisms

PAO are modelled as a single species and GAO are not included in the model. Like ASM2d, the model distinguishes between readily biodegradable organic material in the form of S_{VFA} and more complex soluble, fermentable organic material, S_F. Fermentation is assumed to be facilitated by non-PAO heterotrophic organisms (OHO). For all heterotrophic growth reactions (including PAO), the possibility to use nitrate as a nutrient source when ammonia is limited is included. For $X_{PAO,PP}$, not all of the polyphosphate is considered possible to be released during the phosphate release. Instead, the stored polyphosphate is divided in a releasable and a non-releasable form with separate state variables ($X_{PAO,PP,Lo}$ and $X_{PAO,PP,Hi}$), which is defined by a fixed fraction of the stored orthophosphate that is stored as each state variable. The storage process for $X_{PAO,PP,Lo}/X_{PAO,PP,Hi}$ is considered as part of the growth rate equation for X_{PAO} (which in comparison for ASM2d is included by a separate rate equation) with a stoichiometric relationship. Organically bound (particulate and soluble biodegradable as well as inert soluble) nitrogen is modelled as separate state variables.

The model includes several mechanisms for “loss” of COD in the system, defined as incomplete closure of mass balances, which has been observed in several studies but for which at the time the exact reason for was not known. It is assumed that during fermentation, which includes growth of OHO, a portion of the produced S_{VFA} is in fact not VFA but instead lost from the system. COD loss is also included through the reaction of uptake of S_{VFA} for storage as $X_{PAO,Stor}$, where a yield constant defines the fraction of S_{VFA} that is stored while the remainder is lost from the system. A third mechanism for loss of COD is included in the process of hydrolysis of XC_B to S_F under anoxic or anaerobic conditions, through an efficiency factor. All these processes result in an incomplete COD-balance in the model.

Table 5. Processes included in the Barker & Dold model.

Process #	Process description	Process #	Process description
Ordinary heterotrophic organisms		**Polyphosphate accumulating organisms**	
1	Aerobic growth on S_{F} with S_{NHx}	18	Aerobic growth of X_{PAO} with S_{NHx}
2	Anoxic growth on S_{F} with S_{NHx}	19	Aerobic growth of X_{PAO} with S_{NOx}
3	Aerobic growth on S_{F} with S_{NOx}	20	Aerobic growth of X_{PAO} with S_{NHx} under S_{PO4} limitation
4	Anoxic growth on S_{F} with S_{NOx}	21	Aerobic growth of X_{PAO} with S_{NOx} under S_{PO4} limitation
5	Aerobic growth on S_{VFA} with S_{NHx}	22	Anoxic growth of X_{PAO} on $X_{PAO,Stor}$ with S_{NHx}
6	Anoxic growth on S_{VFA} with S_{NHx}	23	Aerobic decay of X_{PAO}
7	Aerobic growth on S_{VFA} with S_{NOx}	24	$X_{PAO,PP,Lo}$ lysis on aerobic decay
8	Anoxic growth on S_{VFA} with S_{NOx}	25	$X_{PAO,PP,Hi}$ lysis on aerobic decay
9	Decay	26	$X_{PAO,Stor}$ lysis on aerobic decay
Hydrolysis processes		27	Anoxic decay of X_{PAO}
10	Aerobic hydrolysis of XC_{B} to S_{F}	28	$X_{PAO,PP,Lo}$ lysis on anoxic decay
11	Anoxic hydrolysis of XC_{B} to S_{F}	29	$X_{PAO,PP,Hi}$ lysis on anoxic decay
12	Anaerobic hydrolysis of XC_{B} to S_{F}	30	$X_{PAO,Stor}$ lysis on anoxic decay
13	Hydrolysis of organic nitrogen	31	Anaerobic decay of X_{PAO}
14	Ammonification	32	$X_{PAO,PP,Lo}$ lysis on anaerobic decay
15	Fermentation of S_{F} to S_{VFA}	33	$X_{PAO,PP,Hi}$ lysis on anaerobic decay
Autotrophic nitrifying organisms		34	$X_{PAO,Stor}$ lysis on anaerobic decay
16	Aerobic growth of X_{ANO}	35	Cleavage of $X_{PAO,PP,Lo}$ for anaerobic maintenance
17	Decay	36	Sequestration of S_{VFA} by X_{PAO}

3.3 ASM3 + Eawag bio-P module

The ASM3 with Eawag bio-P module (Rieger et al., 2001) builds on the original ASM3 model (see Henze et al., 2000), which was developed for biological nitrogen removal, and extends it to include EBPR. The model contains 17 state variables (Table 6) and 23 processes (Table 7) (compared to 13 state variables and 12 processes in the original ASM3).

PAO are modelled as a single species and GAO are not included in the model. A major difference as compared to ASM2d or the Barker & Dold model is that VFA is not included as a state variable in the model, instead only the total readily biodegradable organic material is included as the state variable S_{B} (which would be the sum of S_{F} and S_{VFA} in the other models). This is motivated by the assumption that there is no limitation on the release of orthophosphate by the fermentation process in municipal wastewater (at normal conditions), meaning that the fermentation process is fast enough to convert fermentable substrate to VFA in the anaerobic zone of the wastewater treatment plant to allow the release of orthophosphate and storage of VFA by PAO at the measured rate. It is therefore assumed that hydrolysis precedes fermentation and is the rate limiting step. It is not clear how valid these assumptions are for situations with higher VFA content than typically measured at the influent of wastewater treatment plants, such as in systems with hydrolysis of primary or secondary sludge (as also pointed out by Hauduc et al. (2013)). The remaining processes are similar to the ones in ASM2d, with the main difference being that endogenous respiration is modelled in ASM3 rather than decay with the death-regeneration concept used in ASM2d (and ASM1). This means that one process for aerobic and one for anoxic endogenous respiration is included, as well as separate aerobic and anoxic processes for lysis of $X_{POA,PP}$ and respiration of $X_{PAO,Stor}$.

Table 6. State variables included in the ASM3 model with Eawag bio-P module, names adapted to standardized notation according to Corominas et al. (2010).

State variable name	Unit	Description
S_{O2}	g O_2.m^{-3}	Dissolved oxygen
S_B	g COD.m^{-3}	Readily biodegradable substrate
S_{NHx}	g N.m^{-3}	Ammonium and ammonia nitrogen
S_{NOx}	g N.m^{-3}	Nitrate and nitrite nitrogen
S_{PO4}	g P.m^{-3}	Ortophosphate phosphorus
S_U	g COD.m^{-3}	Dissolved unbiodegradable organic material
S_{N2}	g N.m^{-3}	Dissolved nitrogen gas
S_{Alk}	mole HCO_3.m^{-3}	Alkalinity
XC_B	g COD.m^{-3}	Slowly biodegradable organic material (particulate and colloidal)
X_U	g COD.m^{-3}	Inert particulate organic material
X_{OHO}	g COD.m^{-3}	Ordinary heterotrophic organisms
$X_{OHO,Stor}$	g COD.m^{-3}	Storage compounds for OHO
X_{PAO}	g COD.m^{-3}	Polyphosphate accumulating organisms
$X_{PAO,PP}$	g P.m^{-3}	Releasable polyphosphate stored in PAO
$X_{PAO,Stor}$	g COD.m^{-3}	Storage products in PAO in the form of PHA
X_{ANO}	g COD.m^{-3}	Autotrophic nitrifying organisms
X_{TSS}	g TSS.m^{-3}	Total suspended solids

Table 7. Processes included in the ASM3 model with Eawag bio-P module.

Process #	Process description	Process #	Process description
Hydrolysis processes		**Autotrophic nitrifying organisms (cont.)**	
1	Hydrolysis	12	Anoxic endogenous respiration
Ordinary heterotrophic organisms		**Polyphosphate accumulating organisms**	
2	Aerobic storage of $X_{OHO,Stor}$	13	Storage of $X_{PAO,Stor}$
3	Anoxic storage of $X_{OHO,Stor}$	14	Aerobic storage of $X_{PAO,PP}$
4	Aerobic growth	15	Anoxic storage of $X_{PAO,PP}$
5	Anoxic growth	16	Aerobic growth
6	Aerobic endogenous respiration	17	Anoxic growth
7	Anoxic endogenous respiration	18	Aerobic endogenous respiration
8	Aerobic respiration of $X_{OHO,Stor}$	19	Anoxic endogenous respiration
9	Anoxic respiration of $X_{OHO,Stor}$	20	Aerobic lysis of $X_{PAO,PP}$
Autotrophic nitrifying organisms		21	Anoxic lysis of $X_{PAO,PP}$
10	Growth	22	Aerobic respiration of $X_{PAO,Stor}$
11	Aerobic endogenous respiration	23	Anoxic respiration of $X_{PAO,Stor}$

3.4 ASM2d + TUD

The ASM2d + TUD metabolic model (Meijer, 2004) is an extension of ASM2d to include a metabolic model for the description of EBPR. The author mentions that this type of model is more consistent and that less model parameters are needed. The model contains 18 state variables (Table 8) and 22 processes (Table 9).

Compared to ASM2d, which uses a grey box approach when modelling the internal cell conversions and instead relies on the observed conversions in the bulk liquid, the metabolic model includes all internal storage compounds (PHA, glycogen and polyphosphate). Model yields are based on the metabolic conversions that are mediated by ATP and $NADH_2$ and included in the model for the calculation of yield for the different PAO reactions (PHA formation, glycogen formation, PHA degradation, etc). The metabolic conversions through ATP/$NADH_2$ are assumed to be in steady state. The reactions for OHO and ANO are identical to ASM2d. Hydrolysis is in the model considered to be mediated by both OHO and PAO, while the original ASM2d only consider OHO as involved in the reaction. The uptake and storage of S_{VFA} is assumed to only take place under anaerobic (by degradation of stored glycogen, $X_{\mathrm{PAO,Gly}}$, and polyphosphate, $X_{\mathrm{PAO,PP}}$) and anoxic (by degradation of $X_{\mathrm{PAO,PP}}$ only, no degradation of $X_{\mathrm{PAO,Gly}}$) conditions, while in ASM2d this process can also occur under aerobic conditions. $X_{\mathrm{PAO,Gly}}$ is formed under aerobic/anoxic conditions by degradation of stored PHA ($X_{\mathrm{PAO,PHA}}$). The lysis of X_{PAO} is replaced with the concept of maintenance, where stored $X_{\mathrm{PAO,PP}}$ is converted to S_{PO4}. The decay of biomass is instead included in the yield constants for the different storage equations, contrary to OHO and ANO for which the death-regeneration system is used.

Table 8. State variables for the ASM2d + TUD metabolic model, names adapted to standardized notation according to Corominas et al. (2010).

State variable name	Unit	Description
S_{O2}	g O_2.m^{-3}	Dissolved oxygen
S_{F}	g COD.m^{-3}	Fermentable, readily biodegradable substrate
S_{VFA}	g COD.m^{-3}	Fermentation products, such as volatile fatty acids (VFA), assumed to be acetate
S_{NHx}	g N.m^{-3}	Ammonium and ammonia nitrogen
S_{NOx}	g N.m^{-3}	Nitrate and nitrite nitrogen
S_{PO4}	g P.m^{-3}	Ortophosphate phosphorus
S_{U}	g COD.m^{-3}	Dissolved unbiodegradable organic material
S_{Alk}	mole HCO_3.m^{-3}	Alkalinity
S_{N2}	g N.m^{-3}	Dissolved nitrogen gas
XC_{B}	g COD.m^{-3}	Slowly biodegradable organic material (particulate and colloidal)
X_{U}	g COD.m^{-3}	Inert particulate organic material
X_{OHO}	g COD.m^{-3}	Ordinary heterotrophic organisms
X_{PAO}	g COD.m^{-3}	Polyphosphate accumulating organisms
$X_{\mathrm{PAO,PP}}$	g P.m^{-3}	Polyphosphate stored in PAO
$X_{\mathrm{PAO,PHA}}$	g COD.m^{-3}	Storage products in PAO in the form of PHA
$X_{\mathrm{PAO,Gly}}$	g COD.m^{-3}	Storage products in PAO in the form of glycogen
X_{ANO}	g COD.m^{-3}	Autotrophic nitrifying organisms
X_{TSS}	g TSS.m^{-3}	Total suspended solids

Table 9. Processes included in the ASM2d + TUD metabolic model.

Process #	Process description	Process #	Process description
Hydrolysis processes		**Polyphosphate accumulating organisms (cont.)**	
1	Aerobic hydrolysis	12	Anoxic storage of S_{VFA}
2	Anoxic hydrolysis	13	Anoxic $X_{PAO,PHA}$ consumption
3	Anaerobic hydrolysis	14	Anoxic storage of $X_{PAO,PP}$
Ordinary heterotrophic organisms		15	Anoxic glycogen formation
4	Aerobic growth on S_F	16	Anoxic maintenance
5	Aerobic growth on S_{VFA}	17	Aerobic $X_{PAO,PHA}$ consumption
6	Anoxic growth on S_F	18	Aerobic storage of $X_{PAO,PP}$
7	Anoxic growth on S_{VFA}	19	Aerobic glycogen formation
8	Fermentation of S_F to S_{VFA}	20	Aerobic maintenance
9	Lysis	**Autotrophic nitrifying organisms**	
Polyphosphate accumulating organisms		21	Aerobic growth of X_{ANO}
10	Anaerobic storage of S_{VFA}	22	Lysis
11	Anaerobic maintenance		

3.5 UCTPHO+

The UTCPHO+ model (Hu et al., 2007) is similar to previously described models in many ways, and contains 16 state variables (Table 10) and 35 processes (Table 11). Key differences from the other models includes: i) hydrolysis of XC_B is not explicitly modelled. Instead, XC_B is transformed to "adsorbed" slowly biodegradable substrate in the activated sludge and then directly used for biomass growth by OHO. This means that S_F is not produced in the model. Release of nutrients is assumed to occur during the heterotrophic growth process; ii) since S_F is not produced in the model, only the influent S_F is available for fermentation by OHO.

Table 10. State variables for the UCTPHO+ model, names adapted to standardized notation according to Corominas et al. (2010).

State variable name	Unit	Description
S_{O2}	g O_2.m^{-3}	Dissolved oxygen.
S_F	g COD.m^{-3}	Fermentable, readily biodegradable substrate.
S_{VFA}	g COD.m^{-3}	Fermentation products, such as volatile fatty acids (VFA), assumed to be acetate.
S_{NHx}	g N.m^{-3}	Ammonium and ammonia nitrogen.
S_{NOx}	g N.m^{-3}	Nitrate and nitrite nitrogen.
S_{PO4}	g P.m^{-3}	Ortophosphate phosphorus.
S_U	g COD.m^{-3}	Dissolved unbiodegradable organic material.
XC_B	g COD.m^{-3}	Enmeshed slowly biodegradable organic material (particulate and colloidal).
X_{Ads}	g COD.m^{-3}	Adsorbed slowly biodegradable organic material.
$X_{U,inf}$	g COD.m^{-3}	Inert particulate organic material from the influent.
$X_{U,E}$	g COD.m^{-3}	Particulate undegradable endogenous products.
X_{OHO}	g COD.m^{-3}	Ordinary heterotrophic organisms.
X_{PAO}	g COD.m^{-3}	Polyphosphate accumulating organisms.
$X_{PAO,PP}$	g P.m^{-3}	Polyphosphate stored in PAO.
$X_{PAO,Stor}$	g COD.m^{-3}	Storage products in PAO in the form of PHA.
X_{ANO}	g COD.m^{-3}	Autotrophic nitrifying organisms.

Table 11. Processes included in the UCTPHO+ model.

Process #	Process description	Process #	Process description
Ordinary heterotrophic organisms		**Polyphosphate accumulating organisms**	
1	Aerobic growth on S_{VFA} with S_{NHx}	18	Aerobic growth of X_{PAO} on $X_{PAO,Stor}$ with S_{NHx}
2	Aerobic growth on S_{VFA} with S_{NOx}	19	Aerobic growth of X_{PAO} on $X_{PAO,Stor}$ with S_{NOx}
3	Anoxic growth on S_{VFA} with S_{NHx}	20	Aerobic growth of X_{PAO} on $X_{PAO,Stor}$ with S_{NHx} under S_{PO4} limitation
4	Anoxic growth on S_{VFA} with S_{NOx}	21	Aerobic growth of X_{PAO} on $X_{PAO,Stor}$ with S_{NOx} under S_{PO4} limitation
5	Aerobic growth on S_F with S_{NHx}	22	Anoxic growth of X_{PAO} on $X_{PAO,Stor}$ with S_{NHx}
6	Aerobic growth on S_F with S_{NOx}	23	Anoxic growth of X_{PAO} on $X_{PAO,Stor}$ with S_{NOx}
7	Anoxic growth on S_F with S_{NHx}	24	Aerobic decay of X_{PAO}
8	Anoxic growth on S_F with S_{NOx}	25	$X_{PAO,PP}$ lysis on aerobic decay
9	Aerobic growth on X_{Ads} with S_{NHx}	26	$X_{PAO,Stor}$ lysis on aerobic decay
10	Aerobic growth on X_{Ads} with S_{NOx}	27	Anoxic decay of X_{PAO}
11	Anoxic growth on X_{Ads} with S_{NHx}	28	$X_{PAO,PP}$ lysis on anoxic decay
12	Anoxic growth on X_{Ads} with S_{NOx}	29	$X_{PAO,Stor}$ lysis on anoxic decay
13	Adsorption of XC_B	30	Anaerobic decay of X_{PAO}
14	Decay	31	$X_{PAO,PP}$ lysis on anaerobic decay
15	Conversion of S_F to S_{VFA} (fermentation)	32	$X_{PAO,Stor}$ lysis on anaerobic decay
Autotrophic nitrifying organisms		33	Cleavage of $X_{PAO,PP}$ for anoxic maintenance
16	Aerobic growth of X_{ANO}	34	Cleavage of $X_{PAO,PP}$ for anaerobic maintenance
17	Decay	35	Sequestration of S_{VFA} by X_{PAO}

3.6 ASM2d-N_2O

Massara et al. (2018) developed an extension of the ASM2d model to include the production of N_2O (a powerful greenhouse gas) in the treatment process. The model contains 24 state variables (Table 12) and 40 processes (Table 13).

Three N_2O production pathways are included in the model: i) NH_2OH oxidation pathway, meaning that the nitrifiers are modelled as two separate species with ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB); ii) nitrifier denitrification pathway (by AOB); iii) heterotrophic denitrification pathway. This means that several intermediate compounds in the nitrification and denitrification is modelled, with nitrification divided in 6 steps (5 by AOB) and denitrification in 4 steps. The 4 steps for denitrification are also included for PAO. Stripping of N_2O from the liquid to the atmosphere is included through the volumetric mass transfer coefficient (k_La) for N_2O.

Table 12. State variables for ASM2d-N_2O, names adapted to standardized notation according to Corominas et al. (2010).

State variable name	Unit	Description
S_{O2}	g O_2.m^{-3}	Dissolved oxygen
S_F	g COD.m^{-3}	Fermentable, readily biodegradable substrate
S_{VFA}	g COD.m^{-3}	Fermentation products, such as volatile fatty acids (VFA), assumed to be acetate
S_{NHx}	g N.m^{-3}	Ammonium and ammonia nitrogen
S_{NH2OH}	g N.m^{-3}	Hydroxylamine nitrogen
S_{N2O}	g N.m^{-3}	Nitrous oxide nitrogen
S_{NO}	g N.m^{-3}	Nitric oxide nitrogen
S_{NO2}	g N.m^{-3}	Nitrite nitrogen
S_{NO3}	g N.m^{-3}	Nitrate nitrogen
S_{PO4}	g P.m^{-3}	Ortophosphate phosphorus
S_U	g COD.m^{-3}	Dissolved unbiodegradable organic material
S_{Alk}	mole HCO_3.m^{-3}	Alkalinity
S_{N2}	g N.m^{-3}	Dissolved nitrogen gas
XC_B	g COD.m^{-3}	Slowly biodegradable organic material (particulate and colloidal)
X_U	g COD.m^{-3}	Inert particulate organic material
X_{OHO}	g COD.m^{-3}	Ordinary heterotrophic organisms
X_{PAO}	g COD.m^{-3}	Polyphosphate accumulating organisms
$X_{PAO,PP}$	g P.m^{-3}	Polyphosphate stored in PAO
$X_{PAO,Stor}$	g COD.m^{-3}	Storage products in PAO in the form of PHA
X_{AOO}	g COD.m^{-3}	Ammonia oxidizing organisms
X_{NOO}	g COD.m^{-3}	Nitrite oxidizing organisms
X_{TSS}	g TSS.m^{-3}	Total suspended solids
X_{MeOH}	g MeOH.m^{-3}	Metal-hydroxides
X_{MeP}	g MeP.m^{-3}	Metal-phosphates

Table 13. Processes included in ASM2d-N_2O.

Process #	Process description	Process #	Process description
Hydrolysis processes		**Polyphosphate accumulating organisms (cont.)**	
1	Aerobic hydrolysis of XC_B to S_F	21	Anoxic storage of $X_{PAO,PP}$ (3rd step: $S_{NO} \rightarrow S_{N2O}$)
2	Anoxic hydrolysis of XC_B to S_F (1st step: $S_{NO3} \rightarrow S_{NO2}$)	22	Anoxic storage of $X_{PAO,PP}$ (4th step: $S_{N2O} \rightarrow S_{N2}$)
3	Anoxic hydrolysis of XC_B to S_F (2nd step: $S_{NO2} \rightarrow S_{NO}$)	23	Aerobic growth of X_{PAO}
4	Anaerobic hydrolysis of XC_B to S_F	24	Anoxic growth of X_{PAO} (1st step: $S_{NO3} \rightarrow S_{NO2}$)
Ordinary heterotrophic organisms		25	Anoxic growth of X_{PAO} (2nd step: $S_{NO2} \rightarrow S_{NO}$)
5	Aerobic growth on S_F	26	Anoxic growth of X_{PAO} (3rd step: $S_{NO} \rightarrow S_{N2O}$)
6	Aerobic growth on S_{VFA}	27	Anoxic growth of X_{PAO} (4th step: $S_{N2O} \rightarrow S_{N2}$)
7	Anoxic growth on S_F (1st step: $S_{NO3} \rightarrow S_{NO2}$)	28	Lysis of X_{PAO}
8	Anoxic growth on S_F (2nd step: $S_{NO2} \rightarrow S_{NO}$)	29	Lysis of $X_{PAO,PP}$
9	Anoxic growth on S_F (3rd step: $S_{NO} \rightarrow S_{N2O}$)	30	Lysis of $X_{PAO,Stor}$
10	Anoxic growth on S_F (4th step: $S_{N2O} \rightarrow S_{N2}$)	**Autotrophic nitrifying organisms**	
11	Anoxic growth on S_{VFA} (1st step: $S_{NO3} \rightarrow S_{NO2}$)	31	S_{NHx} oxidation to S_{NH2OH} coupled with S_{O2} consumption
12	Anoxic growth on S_{VFA} (2nd step: $S_{NO2} \rightarrow S_{NO}$)	32	S_{NH2OH} oxidation to S_{NO} coupled with S_{O2} reduction (with X_{AOO} growth)
13	Anoxic growth on S_{VFA} (3rd step: $S_{NO} \rightarrow S_{N2O}$)	33	S_{NO} oxidation to S_{NO2} coupled with S_{O2} reduction
14	Anoxic growth on S_{VFA} (4th step: $S_{N2O} \rightarrow S_{N2}$)	34	S_{NO} reduction to S_{N2O} coupled with the S_{NH2OH} oxidation to S_{NO2} (N_2O from the NH_2OH oxidation pathway)
15	Fermentation of S_F to S_{VFA}	35	S_{NO2} reduction to S_{N2O} coupled with S_{NH2OH} oxidation to S_{NO2} (N_2O from the nitrifier denitrification pathway)
16	Lysis	36	Aerobic growth of X_{NOO}
Polyphosphate accumulating organisms		37	Lysis of X_{AOO}
17	Storage of $X_{PAO,Stor}$	38	Lysis of X_{NOO}
18	Aerobic storage of $X_{PAO,PP}$	**Simultaneous precipitation**	
19	Anoxic storage of $X_{PAO,PP}$ (1st step: $S_{NO3} \rightarrow S_{NO2}$)	39	Precipitation
20	Anoxic storage of $X_{PAO,PP}$ (2nd step: $S_{NO2} \rightarrow S_{NO}$)	40	Redissolution

3.7 Sumo EBPR model

After studies showing that current commercial EBPR models underestimated the EBPR performance in sidestream hydrolysis configurations (Carson, 2012; Dunlap et al., 2016), Varga et al. (2018) developed a new biokinetic model for EBPR with the goal of it being applicable for both regular EBPR and sidestream hydrolysis configurations without major parameter changes. The model is implemented in the simulation software Sumo and exists in several versions (e.g. Sumo1 with 1-step nitrification and Sumo2 with 2-step nitrification and denitrification). Due to the extensive number of processes and state variables it is not reproduced here.

The model has been developed as an extension of the Barker & Dold model (Barker & Dold, 1997) with addition of a single species of GAO, which have a competitive advantage over PAO at higher temperatures. Microbial maintenance reactions are added for GAO and PAO so that storage pools are depleted before any biomass decay begins. Storage of glycogen is considered only for GAO and is inhibited during low ORP conditions to suppress GAO growth (to account for observations of low abundance of GAO in such systems). PAO are also able to ferment under low ORP conditions to simulate the impact of *Tetrasphaera* while avoiding adding a separate PAO species to the model. The releasable and non-releasable stored polyphosphate in PAO from the Barker & Dold model is changed to allow for complete depletion of the polyphosphate storage pool.

3.8 META-ASM

The META-ASM (Santos et al., 2020) is a metabolic model that was developed to address known issues of EBPR-models. They state that literature models lack detailed understanding of the underlying processes and therefore require substantial changes from the default parameter values, affecting and compromising the predictive power of the models. The META-ASM therefore aims to describe EBPR with a robust set of default parameter values that does not require adjustment depending on the type of process configurations (i.e. conventional or side stream EBPR). The overall model is based on the ASM-inCTRL model (inCTRL Solutions Inc, Ontario, Canada) (which in turn is based on the Barker & Dold model (Barker & Dold, 1997)) while incorporating the EBPR related processes from Lopez-Vasquez et al. (2009), Oehmen et al. (2010) and Lanham et al. (2014). The model contains 41 state variables and 109 processes. The full stoichiometric matrix has not been published, so the state variables and processes are not reproduced here.

The model includes one PAO species (*Ca.* Accumilibacter, meaning that *Tetrasphaera* are not included) while including two separate species of GAO. All PAO can denitrify with nitrite, while only a fraction can denitrify with nitrate; both GAO species have subgroups that can and cannot denitrify. PAO have a competitive advantage over GAO during extended starvation conditions (such as with RAS fermentation) due to higher energy requirement for GAO. The effect of availability of different carbon sources and DO concentration on the competition between PAO and GAO is also included as acetate and propionate are included separately and PAO have a competitive advantage at low DO levels due to higher oxygen affinity.

The model includes shifts between metabolic pathways for PAO which allows modelling of dynamic shifts of the yields of PHA formation and PO_4 release observed in reality. PAO energy generation is considered through both intracellular poly-P hydrolysis as well as glycogen degradation. The decay process is modelled in sequence, where PHA, glycogen and polyphosphate reserves are depleted before the biomass starts to decay.

3.9 Lindstrøm Sørensen et al. model

Lindstrøm Sørensen et al (2019) designed a model for prediction of PO_4-P concentration in the effluent of a wastewater treatment plant, drawing inspiration from both ASM2 (Gujer et al., 1995) and a similar model for nitrogen removal developed by Stentoft et al. (2019). The model is a hybrid data-driven/mechanistic, based on coupled stochastic differential equations and contain only three state variables: oxygen saturation (value ranging from 0-1) as well as PO_4-P concentration in the influent and in the effluent. Some aspects of ASM2 are included, such as Monod kinetics, and to be able to use this without including the biomass concentration as a state variable it is assumed constant over each evaluation period. An adaptive model structure is used to update the state values before each simulation period based on the previous performance through an Extended Kalman Filter. Input data for the model include online sensor data of the effluent PO_4-P concentration, oxygen setpoint for aeration and influent flow rate. For each evaluation period, the model is used to predict the future effluent PO_4-P concentration (24 hours ahead) and is used for Model Predictive Control by optimising aeration input.

3.10 Model comparison

A summary of key differences between the mechanistic models presented above is shown in Table 14.

Table 14. Comparison between several key aspects of the mechanistic models considered in this review, including: number of PAO and GAO species; inclusion of VFA as a state variable, and if distinction is made between different VFAs (e.g. acetate (Ac^-), propionate (Pr^-), etc.); if glycogen storage is considered for PAO and/or GAO; if stored $X_{POA,PP}$ can be fully depleted; if PAO storage of is described by a separate rate equation or is part of the PAO growth rate; if PAO are able to ferment; if OHO fermentation is included as a growth process; and if S_{VFA} uptake can occur under aerobic conditions.

Model	**# of PAO species**	**# of GAO species**	**S_{VFA} included**	**Distinction between VFAs**	**Glycogen storage**	**Full depletion of $X_{POA,PP}$**	**$X_{POA,PP}$ part of biomass**	**PAO fermentation**	**Fermentation growth**	**Aerobic S_{VFA} uptake**
Barker & Dold	1	0	Yes	No	No	No	Yes	No	Yes	No
ASM2d	1	0	Yes	No	No	Yes	No	No	No	Yes
ASM3 + Eawag bio-P module[1]	1	0	No	-	No	Yes	No	-	-	Yes
ASM2d + TUD	1	0	Yes	No	Yes	Yes	Yes	No	No	No
UCTPHO+	1	0	Yes	No	No	Yes	Yes	No	No	No
ASM2d-N_2O	1	0	Yes	No	No	Yes	No	No	No	Yes
Sumo EBPR model	1	1	Yes	No	GAO	Yes	Yes	Yes	Yes	Yes
META-ASM	1	2	Yes	Ac^- & Pr^-	PAO and GAO	Yes	Yes	No	Yes	No

[1] S_{VFA} is not included in the model, thus no fermentation processes either. Aerobic S_{VFA} uptake refers to uptake of S_B for this model.

4 Modelling primary sludge fermentation

The purpose of primary sludge fermentation is to produce VFA for utilization by PAO in the anaerobic zone in the activated sludge process, thus mitigating issues with low influent VFA levels. It incorporates two processes in sequence: hydrolysis of particulate organics to smaller molecules and further fermentation of those molecules to VFA. These processes are already included in most of the models presented above since the processes occur in the activated sludge process, but few published examples of the accuracy of the models for primary sludge hydrolysis and fermentation have been found.

4.1 Hydrolysis

In the ASM model family (Henze et al., 2000), hydrolysis is modelled as a first order-process with respect to heterotrophic biomass concentration, with switching functions for the availability (or absence) of electron donor and the ratio of slowly biodegradable substrate to heterotrophic organisms. The mathematical rate equation for aerobic conditions is shown in Equation 1, the equations for anoxic and anaerobic conditions are similar but contains additional switching functions for substrate and inhibition. Hauduc et al. (2013) mention hydrolysis as one of the few processes where parameter changes from the default values often is required in practice.

$$q_{XCB_{SB},hyd} * \frac{S_{O2}}{K_{O2,hyd} + S_{O2}} * \frac{XC_B/X_{OHO}}{K_{XCB,hyd} + XC_B/X_{OHO}} * X_{OHO} \tag{1}$$

where $q_{XCB_{SB},hyd}$: maximum specific hydrolysis rate; $K_{O2,hyd}$: half-saturation coefficient for aerobic hydrolysis; $K_{XCB,hyd}$: half-saturation coefficient for XC_B/X_{OHO}. The other variables as previously defined.

In the model Anaerobic Digestion Model No. 1 (ADM1) (Batstone et al., 2002), hydrolysis is modelled separately for carbohydrates, proteins and lipids, although each rate equation is a simple first-order process with respect to each variable concentrations. This step is preceded by a disintegration step, where complex organic matter (modelled as a composite variable X_C) is transformed to carbohydrates, proteins and lipids. Rosén et al. (2006) point out that an issue of the original ADM1 model is the use of the composite variable X_C, and therefore it is not included in the conversion of state variables for primary and secondary sludge from ASM1 to ADM1 in the Benchmark Simulation Model No. 2 (Jeppsson et al., 2007). Instead, the state variables are directly converted to lipids, carbohydrates and proteins with different degradation kinetics. Yasui et al. (2008) also states that the simplifications in ADM1 and use of the composite variable was inadequate for detailed description of the hydrolysis of primary sludge and proposed the same change. This gave improvements in model results but increased the complexity of the model considerably. On the other hand, Efstathiou et al. (2019) used an unmodified, calibrated ADM1 to successfully simulate the hydrolysis of primary sludge.

Seco et al. (2004) presented a so called "super model" structure, where a single set of state variables and processes are used for all unit processes, such as activated sludge and anaerobic digestion. They use Equation 1 to describe hydrolysis while also extending it to include hydrolysis by acidogenic bacteria.

4.2 Fermentation

In the ASM2d-model (Henze et al., 1999), the rate equation for fermentation of S_F to S_{VFA} is first-order to the OHO concentration, using a maximum specific fermentation rate ($q_{SF,VFA,Max}$) as well as switching functions for availability of S_F and S_{Alk} and inhibition by S_{O2}/S_{NOx}, according to Equation 2:

$$q_{SF_{VFA},Max} * \frac{K_{O2,OHO}}{K_{O2,OHO} + S_{O2}} * \frac{K_{NOx,OHO}}{K_{NOx,OHO} + S_{NOx}} * \frac{S_F}{K_{fe} + S_F} * \frac{S_{Alk}}{K_{Alk,OHO} + S_{Alk}} * X_{OHO} \qquad (2)$$

The fermentation is thus only performed by OHO in ASM2d, while some of the other models include fermentation by PAO to include the effect of fermenting PAO such as *Tetrasphaera*. It is not clear how well these models describe fermentation of primary sludge without addition of biomass from, for example, the waste activated sludge stream. Characterisation of the influent active biomass COD is therefore probably required, although otherwise neglected in some characterisation methods (Roeleveld & van Loosdrecht, 2002).

Münch, Keller et al. (1999) presented a simple model for primary fermentation. The model assumes a (totally mixed) fermenter followed by a settler to separate solids retention time from hydraulic retention time. The settling process is not explicitly modelled though, instead, the SRT and HRT are used as model parameters. Hydrolysis of unsoluble organics is modelled similar to anaerobic hydrolysis in the ASMs, while including many equations from anaerobic digestion models for the remaining processes. The model was tested in case studies and performed well in dynamic prediction of pre-fermentation (Münch, Lant, et al., 1999).

Ribes et el. (2002) proposed a model suitable for primary sludge fermentation in the primary settler, where hydrolysis and fermentation occur in the sludge blanket of the settler after which the sludge is recirculated to the inlet of the settler. While the sludge settles once again, the VFA that has been produced follows the water flow to the next stage of the treatment plant. The model is based on the settling model presented by Takács et al. (1991), extended to include a compression settling term according to Härtel & Pöpel (1992). It also includes biological reactions according to ASM2d (Henze et al., 1999) extended with a subgroup of heterotrophic bacteria, acidogens, that performs fermentation of S_F to S_{VFA} as a growth process rather than fermentation being mediated by OHO. This switch is also justified by Seco et al. (2004) on the basis that the literature values of $q_{SF_{VFA},Max}$ are highly variable when they are dependent on X_{OHO} as in ASM2d, which can be solved by introducing this new group of anaerobically fermenting heterotrophic organisms.

5 Issues identified with current process models

Following good modelling practice for activated sludge models (Rieger et al., 2013), biokinetic parameters should not be adjusted from the default values presented in the publications unless there is valid experimental support for the change. Rieger et al. further advice the users of the EBRP models ASM2d/ASM3 + Eawag bioP to exercise extra caution when changing the default values since several processes are linked but described separately, such as that the biomass is described separately from their internal storage compounds. This means that if one is changed there is a risk that the mass balances are no longer correct. At the same time, they note that "for ASM2d no widely accepted default parameter set has been published". The difficulties with obtaining a single parameter set that is valid for a wide range of configurations and process conditions in ASM2d can be an artefact of the simplification of modelling only one PAO species and no GAO species. The GAO activity is therefore included in the parameters describing PAO processes, which must be changed with the relative abundance of PAO vs GAO. Systems with higher GAO presence can thus require larger changes in the parameter values. This can be a contributing reason for why the side stream processes such as RAS fermentation are not well described by most of the models described here using default values (the issue that the Sumo EBPR model and the META-ASM were developed to address), as was described in Section 3.7 and 3.8.

Hauduc et al. (2013) performed a review and comparison of several activated sludge models, where the following issues/different modelling approaches relevant to EBPR modelling were highlighted:

- Many models neglect the growth of OHO during the fermentation process, which can have an impact on the results in some situations although the yield is low (0.10 g COD.g COD^{-1} (Ekama & Wentzel, 1999)).
- A COD loss has been observed during anaerobic processes (Barker & Dold, 1995), which was modelled as H_2 formation by Kraemer et al. (2008). This is not included in several of the models, which can result in faulty oxygen consumption and sludge production.
- Sometimes part of the stored polyphosphate cannot be released, perhaps due to glycogen depletion (Brdjanovic et al., 1998), which is only captured by some of the models (e.g. Barker & Dold).
- ASM3 + Eawag bioP assumes that fermentation is not a rate limiting step and ignores the fermentation process, which might cause issues if the process is rate limited by fermentation.
- Inclusion of GAO species could improve prediction of EBPR process disturbances sometimes observed. As of now, only a few models include GAO species.
- The ratio of phosphorus released to VFA uptake changes with pH (Mino et al., 1998), which many models neglect.
- PAO direct growth on VFA under aerobic conditions is neglected in many models, but has been observed in reality (Wentzel et al., 1989). If VFA reaches the aerobic zone, the model can thus produce faulty results.

Acknowledgements

This document has been produced during the project *Digital twin for sustainable and resource efficient operation of wastewater treatment plants*, funded by the Swedish Research Council Formas (2020-00222) as well as the utility organization Nordvästra Skånes Vatten och Avlopp (NSVA) AB and The Swedish Water and Wastewater Association (through the research cluster VA-kluster Mälardalen).

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