Modelling biological nutrient removal activated sludge systems—a review
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Abstract

The external nitrification (EN) biological nutrient removal (BNR) activated sludge (ENBNRAS) system shows considerable promise for full-scale implementation. As an aid for this implementation, a mathematical simulation model would be an invaluable tool. To develop such a model, a study was conducted to select the most suitable simulation model to serve as a starting point for further development. For this, the existing available simulation models for BNRAS systems are compared with one another and evaluated against experimental observations in the literature and on ENBNRAS systems. One process immediately apparent to be crucially important is the anoxic growth of phosphorus accumulating organisms (PAOs), with associated PAO denitrification and anoxic P uptake for polyP formation. These linked processes are lacking in the earlier kinetic simulation models for BNRAS systems, which were based on aerobic PAO growth and P uptake only, but have been incorporated into the more recent kinetic models. This provides a substantive body of information on modelling this aspect. Other processes of significance identified to require consideration are anaerobic slowly biodegradable COD (SBCOD) hydrolysis to readily biodegradable COD (RBCOD), and COD loss. Both processes have significant impact on the predicted BEPR performance. Due to the uncertainties associated with the mechanisms and quantification of these two processes, it is concluded that the most extensively validated kinetic simulation model should be selected for development, and that the omissions in this model should be addressed progressively, using the relevant information drawn from the existing models, the literature and observations on ENBNRAS systems.

Keywords: Modelling; Biological nutrient removal; External nitrification; Biological excess phosphorus removal; Anoxic P uptake

1. Introduction

In the external nitrification (EN) biological nutrient removal (BNR) activated sludge (ENBNRAS) system, the nitrification process is removed from the main BNRAS system to a fixed media system [1–4]. From an extensive laboratory-scale investigation into this system, Hu et al. [5] concluded that the system provides opportunity for substantial BNRAS system intensification, and holds considerable promise for full-scale implementation, particularly at plants with existing trickling filters and conventional BNRAS systems. To aid the design, operation and optimisation of, and further research into this system, a mathematical simulation model would be invaluable as a process evaluation tool. Accordingly, it was decided to develop such a model. The approach taken in this model development was to first evaluate and compare the available existing kinetic models for BNRAS systems, and then to select the most suitable for further development. This paper describes this evaluation.

State of the art BNRAS system kinetic models identified for evaluation are:

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• The IAWQ (now IWA) activated sludge model no. 2 (ASM2) [7], and
• The IAWQ activated sludge model no. 2d (ASM2d) [8],
• The Barker and Dold [9] model (called BIOWIN in implementation in computer programmes),
• The Delft based research group models [10–16].

In the initial assessment of these models and the results from ENB-NRAS systems, one process immediately apparent to be crucially important was the anoxic P uptake and associated denitrification by phosphate accumulating organisms (PAOs), and thus attention is focused on this aspect; anoxic P uptake and associated PAO denitrification are features endemic to the EN-BNARAS system [5]. In the model evaluation, it is not possible to present all aspects of the various models, but mainly those aspects particularly relevant to biological N and P removal will be discussed.

2. Development of kinetic models for BNRAS systems

2.1. The UCTOLD [17] and ASM1 [18] models

To promote the development of mathematical models for biological wastewater treatment systems and to facilitate their practical application in design and operation, in 1983 the IAWPRC (subsequently IAWQ, now IWA) set up a Task Group on Mathematical Modelling for Design and Operation of Biological Wastewater Treatment. To develop a mathematical model for activated sludge systems, the Task Group undertook a wide-ranging survey of research contributions. One contribution identified of importance by the Task Group was the kinetic model developed by Dold et al. [19] for aerobic systems and extended to include denitrification by Van Haandel et al. [20], which consolidated much of the previous research into activated sludge modelling. This University of Cape Town (UCT) based research group model is termed UCTOLD [17].

Based on UCTOLD, the Task Group proposed a general kinetic model for organic matter and nitrogenous material removal, called activated sludge model no. 1, ASM1 [18]. Both models are very similar, and can predict oxygen demand, sludge production, nitrification and denitrification for activated sludge systems, i.e. COD and N removal from the wastewater. In summary, both models include growth and death processes for two groups of organisms: (1) ordinary heterotrophic organisms (OHOs) and (2) autotrophic nitrifying organisms (ANOs), and hydrolysis of particulate slowly biodegradable organics.

Modelling of the ANO growth and associated processes is the same for both models: The ANOs grow by oxidising ammonia to nitrate (or nitrite) via Monod kinetics only under aerobic conditions, and their relative contribution to sludge production is very small (due to their low growth yield and the relatively low TKN/COD ratio of domestic wastewaters). In both models, the OHOs utilise two kinds of biodegradable organics (quantified via the COD), soluble readily biodegradable (RBCOD) and particulate slowly biodegradable (SBCOD) under both aerobic and anoxic conditions. The OHO growth processes on RBCOD are the same for the two models, with growth modelled via Monod kinetics. However, utilisation of SBCOD is modelled significantly differently in UCTOLD and ASM1:

• In UCTOLD, the SBCOD is first enmeshed in the sludge mass (assumed to be instantaneous), then the enmeshed COD is adsorbed onto the OHO surface in accordance with a saturation type kinetic rate. On the OHO surface, it is hydrolysed according to a Monod saturation type rate (also called surface saturation kinetics) in terms of the ratio of adsorbed COD to OHO active biomass. The hydrolysis products produced are immediately and directly utilised by the OHOs,

• In ASM1, the SBCOD is enmeshed in the sludge mass (also assumed to be instantaneous), where it is hydrolysed (no adsorption process) in accordance to the same kinetics in UCTOLD. However, in contrast to UCTOLD, the hydrolysis products are RBCOD which is returned to the bulk liquid where it adds to the RBCOD from the influent. Hence, in effect in ASM1 only RBCOD is utilised by OHOs for growth.

This difference was carried over into the subsequent extensions of the models for BNARAS systems, respectively (i.e. from UCTOLD to UCTPHO, and from ASM1 to ASM2 and 2d, see below).

For the decay/death processes of the OHOs and ANOs, the death-regeneration concept is adopted in both UCTOLD and ASM1: For the OHOs, a fraction (62%, \( b_H = 0.62/d \)) of the OHOs die per day, and release all their organic content to the bulk liquid. Of this, a fraction (8%, \( f_{EH} = 0.08 \)) is unbiodegradable particulate organics and adds to the endogenous residue, while the remainder (1 − \( f_{EH} = 0.92 \)) is biodegradable and adds to the SBCOD, and is recycled through the same processes of enmeshment, hydrolysis and utilisation as the influent SBCOD. For the ANOs, the same concept applies, except that their decay/death rate is very low (\( b_A = 0.05/d \)). Since ANO death generates SBCOD and endogenous residue, this in effect adds COD into the system, which, if not taken into account, will result in >100% COD balances over the system. Since the UCTOLD and ASM1 models were developed, they have achieved wide application in system design, operation and process optimisation of nitrification denitrification activated sludge (NDAS) systems.
2.2. The UCTPHO model

The increased implementation of biological excess P removal (BEPR) during the last two decades created a need to extend UCTOLD and ASM1 to include BEPR. Recognizing the need for a kinetic model describing BNRAS systems, the research group at the University of Cape Town set about extending the UCTOLD model to include BEPR. The BEPR is mediated by a group of heterotrophic organisms (the PAOs) that exhibit the propensity to store P internally as polyphosphate (polyP). Proposed explanations of biochemical behavioural patterns associated with the anaerobic P release and aerobic (or anoxic) P uptake leading to BEPR had been presented in a number of biochemical models [21–24], and these were used as a basis for the kinetic model development [6].

To obtain quantitative kinetic information on BEPR, Wentzel et al. [25] developed PAO enhanced cultures in continuous-flow activated sludge systems (3-stage Bardenpho and UCT configurations) with acetate as the only organic substrate; these enhanced cultures exhibited dominantly aerobic P uptake behaviour, >90%. Based on observations on these systems and batch tests on mixed liquor harvested from these systems, Wentzel et al. [26,27] developed a kinetic BEPR model for aerobic P uptake PAO enhanced culture systems. This model, with a single set of kinetic and stoichiometric constants, provided a good quantitative description of the observed responses in the four constant flow and load systems and in the batch tests on sludges harvested from them [27].

Wentzel et al. [6] integrated the PAO enhanced culture kinetic model for aerobic uptake BEPR into the UCTOLD model to form a general kinetic model for BNRAS systems, termed UCTPHO. This model incorporates three population groups. In addition to the ANOs and OHOs, which are unable to accumulate P as polyP, PAOs which are able to accumulate P as polyP are included. The PAOs take up short-chain fatty acids (SCFA) and store these intracellularly as polyhydroxyalkanoates (PHA) under anaerobic (zero nitrate and oxygen) conditions, releasing P cleaved from polyP in the process. The PHAs are utilised by the PAOs in the subsequent aerobic reactor as substrate with concomitant P uptake for polyP formation (anoxic conditions are considered later). The OHOs, ANOs and PAOs mediate all biological processes for COD removal, nitrification, denitrification and BEPR (see [6]). To model the behaviour of these three organisms groups in the activated sludge system environment, the UCTPHO model follows:

1. The UCTOLD kinetic model for OHOs and ANOs [17,19,20], in combination with
2. (3) the interactions between the OHOs, ANOs and PAOs, such as acid fermentation in the anaerobic reactor of fermentable RBCOD (F-RBCOD) to short chain fatty acid (SCFA) mediated by OHOs, with the resultant SCFA available to PAOs [6,29].

The UCTPHO model was logically structured in the matrix format approach (recommended by the IWA Task Group), with all fundamental conversion processes presented and all compounds (components) involved in the conversion processes (various substrates, nutrients, and organisms) related through the stoichiometry and process kinetic rate equations. This model includes 25 processes and 19 components: Processes 1–12 and 25 reflect OHO behaviour, Processes 13–14 ANO behaviour and Processes 15–24 PAO behaviour (see [6]).

2.2.1. Growth and decay/death processes for the OHOs and ANOs

For the OHOs and ANOs, the processes (\(j = 1–14\), where \(j\) refers to the process number in [6]), their rate formulations and stoichiometric interactions with the compounds remain the same as for the UCTOLD model [17], i.e. the death regeneration-bisubstrate synthesis model, with the exception that the interactions between these processes and the compound phosphorus (P) are added, i.e.:

1. Stoichiometric P requirement for OHO and ANO growth,
2. P-limit switching functions to ensure the growth processes operate only if P is available,
3. stoichiometric P release on OHO and ANO death/decay.

In addition, an extra process was included to take account of OHO behaviour under anaerobic conditions, namely:

4. The conversion (fermentation) process \((j = 25)\) of “complex” or fermentable RBCOD to SCFA by OHOs under anaerobic conditions with the resultant SCFA available to PAOs; the kinetics and stoichiometry for this process were taken unmodified from [29].

From a comparison of simulation results with experimental data [6], it appeared that the values for the kinetic and stoichiometric constants from UCTOLD could be kept unchanged for UCTPHO, with one exception:

5. The value for the ratio of the OHO hydrolysis/ utilisation rate for adsorbed SBCOD under anoxic conditions to aerobic conditions (\(\eta_G\)). The value for \(\eta_G\) had to be increased from 0.33 in UCTOLD to approximately 0.6 in UCTPHO [6] to reflect the experimentally observed denitrification by OHOs in
BNRAS systems [30]. Simulations using the UCTPHO model with the increased \( n_G = 0.6 \) correctly reflected the increased \( K_2 \) and \( K_3 \) specific denitrification rates of 2.5 and 1.5 times, respectively, in the primary and secondary anoxic reactors of BNRAS systems, respectively, compared with the \( K_2 \) and \( K_3 \) rates in NDAS systems [31].

2.2.2. Growth and decay/death processes for the PAOs

For the PAOs, the processes \((j = 15–24)\), the process rate formulations and kinetic constants remain the same as in the aerobic \( P \) uptake PAO enhanced culture kinetic model [27]. However, it must be mentioned that

1. From experimental observations on batch tests, the PAOs can take up SCFAs and store them as PHA under anaerobic, anoxic and aerobic conditions [27]. However, in BNRAS systems, this process will be operative only under anaerobic conditions, because significant SCFA concentrations are likely to be encountered only under these conditions.

2. Following the experimental observations of Wentzel et al. [26,27] and Clayton et al. [30], the PAOs cannot use nitrate as external electron acceptor (i.e. cannot grow under anoxic conditions), and accordingly “perceive” anoxic conditions as anaerobic. The PAOs can grow only under aerobic conditions (i.e. the model considers only aerobic \( P \) uptake BEPR), and only with internally stored PHA as substrate.

3. A modified endogenous respiration approach is used to deal with PAO mass loss (as distinct from death regeneration for the OHOs). The endogenous mass loss is modelled separately for aerobic \((j = 19)\) and anaerobic/anoxic \((j = 20)\) conditions: Under aerobic conditions, a fraction of the PAO mass lost (biodegradable portion \( 1 - f_{EP,G} - f_{ES,G} = 1 - 0.25 - 0.20 = 0.55 \)) is allocated to oxygen utilisation to account for the maintenance energy requirement, and the balance (unbiodegradable portion \( f_{EP,G} + f_{ES,G} = 0.25 + 0.20 = 0.45 \)) is allocated to endogenous residues (particulate + soluble, respectively); under anaerobic/anoxic conditions, because no suitable electron acceptor (oxygen) is available, the biodegradable fraction of the mass loss adds to the enmeshed SBCOD. For both scenarios the PHA and polyP contents contained in the PAO active mass lost are released to the bulk liquid as enmeshed SBCOD and phosphate, respectively. Additionally, a polyP cleavage process \((j = 23)\) is included to supply the maintenance energy requirement in the absence of oxygen, with the cleaved \( P \) adding to the bulk liquid; this process gives rise to the observed phenomenon of “secondary” \( P \) release.

When the UCTPHO model was proposed, Wentzel et al. [6] noted that a number of aspects in the UCTPHO kinetic model required further attention. One major aspect was anoxic \( P \) uptake and associated denitrification by the PAOs. Although UCTPHO drew information from the biochemical model of Wentzel et al. [23] that recognises and describes PAO denitrification with anoxic \( P \) uptake, at that time insufficient experimental information was available to describe the kinetics of denitrifying PAOs. Furthermore, the PAO enhanced culture systems [26,27] on which the model was based and the mixed culture BNRAS systems of Wentzel et al. [32] and Clayton et al. [30] used for model validation exhibited minimal anoxic \( P \) uptake and associated PAO denitrification behaviour. Hence, the UCTPHO model does not include anoxic PAO growth with associated anoxic \( P \) uptake and PAO denitrification, aspects of particular importance to ENBNRAS systems.

2.3. The ASM2 model

Based on the UCTPHO model and other information in the literature, the IWA Task Group extended ASM1 to include BEPR, to form ASM2 [7]. ASM2 differs from UCTPHO in some aspects.

2.3.1. Growth and decay/death processes for the OHOs and ANOs

With regard to the OHOs and ANOs, ASM2 remains the same as ASM1 with the exceptions that:

1. The interactions between the OHOs, ANOs and the compound \( P \) are included, i.e. the \( P \) requirement for OHO and ANO growth is added by incorporating stoichiometric \( P \) uptake for growth and the \( P \)-liming switching functions for the OHO \((j = 4–7\) where \( j \) refers to the process number as given in [7]) and ANO \((j = 16)\) growth processes, as in the UCTPHO model.

2. The conversion process (called fermentation, \( j = 8 \)) by OHOs of “complex” (or fermentable) RBCOD to SCFA that are available to PAOs under anaerobic conditions is also included, as in UCTPHO. However, the process is modelled with Monod kinetics whereas in UCTPHO it is modelled as first order with respect to “complex” RBCOD and OHO active biomass, based on the experimental observations of Wentzel et al. [29].

3. As in the UCTPHO model, RBCOD in ASM2 is divided into two sub-fractions: SCFA (or fermentation product, \( S_F \)) and fermentable RBCOD (\( S_P \)). In UCTPHO OHO growth on SCFA was not included, because most of the SCFA (either from the influent or fermentation/conversion process) are taken up by the PAOs under anaerobic conditions, so that usually very little remains under subsequent anoxic
and aerobic conditions. In ASM2, growth processes for OHOs on both $S_A$ and $S_F$ are included, for both aerobic ($j = 4$ and 5) and anoxic ($j = 6$ and 7) conditions.

Because ASM2 follows ASM1, the difference in the SBCOD hydrolysis/utilisation between ASM1 and UCTOLD is carried over to ASM2 and UCTPHO, respectively:

(4) In ASM1, SBCOD ($X_s$) is first hydrolysed to RBCOD ($S_s$), which adds to the RBCOD bulk liquid ($S_s$) pool from the influent, with OHO growth on RBCOD only. The SBCOD hydrolysis is operative under both aerobic ($j = 1$) and anoxic ($j = 2$) conditions, but with a reduction factor ($\eta_{\text{NO3,HYD}}$) to differentiate the various applications of the symbol $\eta_{\text{NO3}}$ in ASM1 and 2, the subscripts will be modified slightly) applied to the aerobic SBCOD hydrolysis rate for anoxic conditions, to describe the lower anoxic SBCOD hydrolysis rate compared with the aerobic SBCOD hydrolysis rate. Furthermore, a separate reduction factor ($\eta_{\text{NO3,G,OHO}}$) is also applied to the growth processes of OHOs on RBCOD, to give a reduced RBCOD utilisation rate under anoxic conditions. Thus, in ASM1 $\eta$ has two values, $\eta_{\text{NO3,G,OHO}} = 0.80$ for anoxic OHO growth and $\eta_{\text{NO3,HYD}} = 0.4$ for anoxic SBCOD hydrolysis. This approach has been carried over to ASM2, with $\eta_{\text{NO3,G,OHO}}$ remaining at 0.8, but $\eta_{\text{NO3,HYD}}$ increased to 0.6 to take account of the increased denitrification rates in BNRAS systems (see above). In UCTOLD and carried over to UCTPHO, OHO growth on and utilisation of SBCOD and RBCOD are separate, as described above. In UCTOLD, no reduction factor is applied to the aerobic OHO growth rate on RBCOD for anoxic conditions, and this is true for UCTPHO also. Experimental investigations do indicate that the RBCOD uptake rate is reduced under anoxic compared with aerobic conditions (e.g. anoxic/aerobic rate $= \eta_{\text{NO3,G,OHO}} = 0.87$ [33]). However, this deficiency in UCTOLD and UCTPHO is of minor consequence, since invariably primary anoxic reactors in NDAS systems are sufficiently large to ensure complete utilisation of the influent RBCOD, i.e. the process is governed by stoichiometry, not kinetics, and in BNRAS systems little influent RBCOD is utilised anoxically, due to the fermentation and SCFA uptake processes occurring in the up-front anaerobic reactor [30]. In any event, the OHO maximum specific growth rate on RBCOD is variable, changing significantly with reactor configuration [33,34].

For SBCOD, in UCTOLD the SBCOD hydrolysis and utilisation processes are modelled as a single process for both aerobic and anoxic conditions, with an anoxic reduction factor ($\eta_{\text{g}} = 0.33$, equivalent to $\eta_{\text{NO3,HYD}}$ in ASM1 and 2) applied to the OHO aerobic rate for anoxic conditions. This approach has been carried over to UCTPHO also, but with $\eta_{\text{g}} = 0.6$. Thus, for SBCOD the difference between the UCTOLD and ASM1 approaches to modelling its hydrolysis/utilisation are carried over to UCTPHO and ASM2, respectively. However, this difference will not influence the predictions significantly, provided the equivalent $\eta$ values for SBCOD hydrolysis are calibrated appropriately in both models, as is the case.

In extending application of the models to systems that include anaerobic zones, a significant difference between the two models in anaerobic SBCOD hydrolysis was introduced:

(5) In ASM2, SBCOD hydrolysis also can take place under anaerobic (absence of terminal electron acceptors oxygen and nitrate) conditions ($j = 3$), with a reduction factor $\eta_{\text{Fe}}$ applied to the aerobic rate for anaerobic conditions. The RBCOD thus produced anaerobically, adds to the fermentable RBCOD ($S_F$) in the bulk liquid, which is the substrate source for the fermentation process producing SCFA ($S_A$), which in turn becomes available to the PAOs for uptake. This implies that SBCOD contributes indirectly to the BEPR. The extent that SBCOD contributes to the BEPR will depend on the value for $\eta_{\text{Fe}}$. If the $\eta_{\text{Fe}}$ value is high, significant SBCOD will be hydrolysed, and hence this will significantly influence BEPR. In ASM2, a low value ($\eta_{\text{Fe}} = 0.1$) is suggested, but there is no experimental basis for this value [35]. In UCTPHO, anaerobic SBCOD hydrolysis is not included as it was considered unlikely that SBCOD contributes significantly to BEPR [35,36].

ASM2 introduced a new more logically structured approach to modelling organic N and P transformations:

(6) In ASM1, as in the UCTOLD and UCTPHO models, two processes model the conversion of organic N to free and saline ammonia (FSA): (a) hydrolysis of biodegradable particulate organic N ($N_{\text{obs}}$) (i.e. the organic N associated with SBCOD) to biodegradable soluble organic N ($N_{\text{obs}}$) by OHOs at a rate proportional to the rate of SBCOD hydrolysis, and then (b) ammonification by the OHOs of the resultant (and influent) $N_{\text{obs}}$ to FSA at an independent rate. In contrast, in ASM2 the transformations between the different N forms are stoichiometrically linked to the corresponding transformations between the organics: Thus, when fermentable RBCOD ($S_F$) is anaerobically fermented to SCFA ($S_A$) or anoxically/aerobically utilised by OHOs for growth, the amount of organic N associated with $S_F$ is released as FSA into the bulk solution, with concomitant FSA uptake for cell synthesis in the growth processes. When SBCOD
(\(X_i\)) is hydrolysed to \(S_F\) by OHOs, the difference between the amounts of organic N associated with \(X_i\) and \(S_F\) is simultaneously released to the bulk solution as FSA. In this manner, the two organic N conversion processes are omitted, and replaced by stoichiometric relationships in the relevant conversion or growth processes on \(S_F\) and the SBCOD hydrolysis. The same approach is followed for the organic P compounds.

With regard to the decay processes for OHOs and ANOs in ASM2, these remain the same as in ASM1 or UCTPHO, i.e. death-regeneration (see above).

2.3.2. Growth and decay/death processes for the PAOs

For the PAOs, ASM2 follows UCTPHO, but with some differences:

1. UCTPHO includes two processes (\(j = 17\) and \(18\) \cite{6}) for PAO growth under P-limiting conditions, while ASM2 does not include these two processes. Thus, ASM2 cannot simulate PAO growth and associated BEPR processes under P-limiting conditions and growth of PAOs ceases under these conditions. To attempt to avoid P limited growth, the growth processes are given preference for P over the polyP accumulation processes, through manipulation of the half saturation constants in the P switching functions.

2. In UCTPHO, when FSA is limiting, nitrate can be used as an alternative N source for PAO growth, which adds two additional PAO growth processes (\(j = 16\) and \(18\)). In contrast, in ASM2 the PAOs can use only FSA as N source for growth, i.e. PAO growth ceases when FSA is limiting. Again, manipulation of the half saturation constants in the FSA switching functions attempts to avoid this situation.

3. In UCTPHO, PAO growth and P uptake for polyP formation are lumped together as a single process with a single rate equation; the difference between the rates for the PAO growth and P uptake are reflected in the stoichiometric parameters. In ASM2, PAO growth (\(j = 12\)) and P uptake for polyP formation (\(j = 11\)) are modelled as separate processes. This difference between the two models does not give different simulation results provided the relevant stoichiometric and kinetic parameters are calibrated appropriately.

4. In UCTPHO, PAO decay is modelled with the endogenous mass loss approach, and the biodegradable portion of the PAO mass lost: (a) goes to oxygen (\(j = 19\)) under aerobic conditions, and (b) adds to the SBCOD pool under anoxic and anaerobic conditions (\(j = 20\)). In ASM2, under all conditions (aerobic, anoxic and anaerobic conditions) it goes to SBCOD (\(j = 13\)). Again, the differences in predictions between two models would be minor provided the constants are appropriately calibrated.

5. UCTPHO includes a maintenance process for PAOs under anaerobic/anoxic conditions (\(j = 23\)) to supply PAO maintenance energy requirements which results in the observed phenomenon of secondary P release (i.e. P release not associated with sequestration of SCFA); ASM2 does not include such a process.

2.4. The metabolic model

Soon after the UCTPHO model was developed by Wentzel et al. \cite{6}, Smolders et al. \cite{13–15} developed a structured metabolic model for PAO behaviour, based on PAO enhanced cultures developed in anaerobic/aerobic sequencing batch reactor (SBR) systems. In the model, all the conversions of the relevant components are described by six independent processes, two for anaerobic conditions and four for aerobic conditions. The stoichiometries for these processes were developed from the relevant well-established biochemical pathways (hence the name metabolic model). The rates for the PAO processes are described by four kinetic equations and two maintenance terms \cite{13,14}.

2.4.1. Anaerobic phase

Under anaerobic conditions, as for UCTPHO and ASM2, PAOs take up acetate and store it as polyhydroxybutyrate (PHB) by utilising energy generated in cleavage of polyP to phosphate that is released into the bulk solution. However, additionally in the metabolic model the energy and reducing equivalents generated in conversion of internally stored glycogen to PHB are used in the acetate uptake; this addition arose from experimental observations clearly demonstrating the consumption of internally stored glycogen under anaerobic conditions in the PAO enhanced cultures (and its formation under subsequent aerobic conditions). Smolders et al. \cite{13,14} derived the stoichiometry for the processes in the anaerobic phase from 4 biochemical pathways:

1. Acetate uptake and storage as PHB: The uptake of acetate requires \(z_1\) mol ATP depending on the pH (\(z_1 = 0 – 0.5\)), the conversion of acetate to acetyl-CoA requires 0.5 mol ATP, and the subsequent conversion to PHB requires 0.25 mol NADH\(_2\), all per C-mol acetic acid.

2. Polyphosphate cleavage for ATP production: For uptake and storage of acetate, ATP is produced by cleavage of polyP. The amount of ATP generated from this cleavage is \(z_2\). Since the hydrolysis of
1 P-mol polyP yields 1 mol ATP and 1 mol phosphate, \( z_2 = 1 \) when assuming that no energy is produced by the release of phosphate from the cell to the bulk liquid.

(3) and (4) **NADH\(_2\) and ATP production**: For the generation of the reducing equivalents (NADH\(_2\)) required to form PHB from acetate, two situations were considered: (i) NADH\(_2\) is produced from the oxidation of some acetate in the TCA cycle, with the ATP produced in the TCA cycle used for the conversion of FADH\(_2\) to NADH\(_2\), and (ii) the NADH\(_2\) is produced from the conversion of 0.5 C-mol glycogen via the Embden–Meyerhof pathway to acetylCoA (subsequently converted to PHB), simultaneously yielding 0.25 mol ATP. From observed stoichiometries, it was concluded that the second option is more feasible.

The four stoichiometries above were incorporated into a single anaerobic process, acetate uptake. Additionally, an anaerobic maintenance process was introduced to supply PAO anaerobic maintenance energy requirements—under anaerobic conditions polyP is cleaved and P released to the bulk solution, which is in agreement with UCTPHO. A single stoichiometric coefficient \( z_1 \) describes the PAO anaerobic processes, all other stoichiometries are derived from the relevant biochemical pathways/reactions.

### 2.4.2. Aerobic phase

Under aerobic conditions, the stoichiometries of PAO metabolism were described by 5 well-established biochemical pathways:

1. **PHB degradation**, represented by the aerobic catabolism of the stored PHB via the TCA cycle,
2. **Oxidative phosphorylation**, which is the production of ATP from NADH\(_2\) generated in PHB degradation,
3. **Biomass synthesis from PHB as substrate**—as for UCTPHO and ASM2, PAO growth occurs only with internally stored PHB as substrate and only under aerobic conditions (anoxic PAO growth was considered in later model developments, see below),
4. **P transport and polyP synthesis**, which gives rise to the aerobic uptake of P and subsequent synthesis of polyP, and
5. **Glycogen production**—a fraction of the PHB utilised is converted to glycogen; this differs from UCTPHO and ASM2 where glycogen is not included and arises from direct experimental observations.

These five biochemically-based stoichiometries were incorporated into 3 aerobic PAO processes, all of which use PHB, namely (i) biomass synthesis, (ii) phosphate uptake and polyP synthesis, and (iii) glycogen formation. Additionally, an (iv) aerobic PAO maintenance process was introduced, with PHB as maintenance energy source. To describe the stoichiometry of the aerobic metabolism, three stoichiometric coefficients were identified, the P/O ratio (\( \delta \)), the polymerisation coefficient (\( K \)) and the transport coefficient (\( \varphi \)).

The values for the anaerobic \( (z_1) \) and aerobic \( (\delta, K \text{ and } \varphi) \) stoichiometric coefficients, and the kinetic rate constants were determined by application of the model to the SBR PAO enhanced cultures.

### 2.5. Comments on the UCTPHO, ASM2 and metabolic models

The UCTPHO model has been evaluated against extensive experimental data on laboratory-scale systems receiving acetate or municipal wastewater as influent. However, this evaluation has been restricted to steady state conditions for the reason that extensive steady state experimental data were available [32], whereas there is a lack of experimental data under cyclic flow and load conditions. From the evaluation, it was found that the model gave a good correlation between observed and predicted results for COD, nitrification, denitrification and BEPR provided the P uptake was predominantly aerobic (>90%). Also, it appeared that the values for constants from the UCTOLD and PAO enhanced culture kinetic models could be retained, with the exception of \( \eta_G \) [6]. The value of \( \eta_G \) was increased from 0.33 in UCTOLD to 0.6 in UCTPHO based on the experimental results of Clayton et al. [30], as described above.

The ASM2 model has not been validated extensively against experimental data. However, since the ASM2 model is based to a large extent on the UCTPHO model, it is possible to calibrate ASM2 to give system predicted responses similar to UCTPHO (e.g. [37]). In application of ASM2, Ekama and Wentzel [35] noted that the anaerobic SBCOD hydrolysis process can significantly influence the simulation results for BEPR: If the reduction factor for anaerobic SBCOD hydrolysis \( (\eta_{Fe}) \) is >0, then a part of the SBCOD is in effect given to the PAOs for PHA storage and P release, with the PHA being available for use by the PAOs for growth and P uptake under subsequent aerobic conditions, i.e. the proportion of the influent biodegradable COD obtained by the PAOs increases. This will increase the mass of PAOs in the system and hence the BEPR. The effect of the proportion of influent biodegradable COD obtained by the PAOs on BEPR is demonstrated by Wentzel and Ekama [38]. Although in ASM2 \( \eta_{Fe} \) for anaerobic hydrolysis is given a low value of 0.1, this does imply that this process is operative under anaerobic conditions, and if \( \eta_{Fe} \) is increased, the process will be
The maintenance concept is used to describe the PAO (phosphorus accumulating organisms) role in BEPR (biological phosphorus removal). The metabolic model is based on well-established biochemical pathways for the processes involved in PAO metabolism. These are used to establish the stoichiometries for the anaerobic and aerobic phases, which are a function of the energetic and electron restrictions expressed by 4 parameters (x1, δ, ε and K). Kinetically, the production of biomass, polyP, and glycogen from PHB are not coupled, but can all proceed independently. In the UCTPHO model, only the rate for PHB consumption is defined with the other processes (e.g. P uptake and polyP formation) stoichiometrically linked to it. Furthermore, in UCTPHO the description of the aerobic phase includes two separate submodels (P-limiting and P-not limiting) with different kinetic parameters.

- Glycogen is included in the metabolic model as a compound separate from PHB and is considered essential for BEPR. In UCTPHO and ASM2, this component is not specifically included, but in effect is lumped together with the PAO active biomass; initially the role of glycogen in BEPR was not realised (UCTPHO) and later it was considered unlikely that glycogen would be limiting in normal operation and so it was excluded for simplicity (ASM2).
- The maintenance concept is used to describe the PAO “death” phase in the metabolic model, whereas the endogenous respiration/lysis concept is followed in the UCTPHO and ASM2 models.

The metabolic model has been tested against experimental data from lab-scale sequencing batch reactor (SBRs) systems receiving acetate (synthetic sewage) as substrate only [13,14]. The experimental data were based on direct observations of the variations in biochemical constituents of the PAOs, such as PHB, glycogen and polyP during selected SBR cycles. This allowed complete validation of the stoichiometry derived for the metabolic model. The model was capable of describing the conversions occurring in the SBR systems at sludge ages ranging from 5 to 20d, and also during start-up conditions. Therefore, it appears that the metabolic model is a very powerful tool that allows mathematical description of the behaviour of a specific group of microorganisms (PAOs) with selected biochemical pathways. However, in application of the metabolic model it should be noted that:

1. In the experimental systems of Smolders et al. [13,14], only acetate was used as substrate fed to the anaerobic phase, and thus mainly PAOs grew in the systems. However, in mixed culture BNRAS systems, i.e. systems receiving real wastewater, other organisms such as OHOs will also be present. The metabolic model does not include OHOs, and thus cannot predict the behaviour of mixed culture systems receiving real wastewater; this deficiency has been addressed in later versions of the model, see below.

2. All experimental systems used to develop the metabolic model of Smolders et al. [13,14] were operated under P-limited conditions (i.e. P concentration in the effluent was zero) while the experimental systems on which the kinetic simulation models of UCTPHO and ASM2 are based were operated under carbon-limited conditions (i.e. the COD in the influent is insufficient for complete removal of the influent P, with the result that the effluent P concentration usually > 3 mg P/l). Thus, the ability of the metabolic model to predict the P removal capacity of the systems was untested, because the amount of P that could be removed by the PAOs was limited by the P available in the influent. Hence, the maximum P content of PAOs could not be determined from these P-limited experimental systems.

In the UCTPHO, ASM2 and Delft models, the PAOs grow only under aerobic conditions, and hence these models are not able to predict P uptake and associated denitrification by PAOs under anoxic conditions. Subsequently, attention has been focussed on this aspect in development of the more recent BNR kinetic models (see below).

3. Recent developments in kinetic models for BNRAS systems

From the discussion above, at the time that the above mathematical models were developed, the observations on BNRAS systems were principally associated with aerobic P uptake BEPR, and thus anoxic P uptake and associated denitrification by PAOs (and associated anoxic PAO growth) were not included. Since 1990, the occurrence of anoxic P uptake has been reported more frequently in lab-scale [10,39] and full-scale systems [12]. Ikma and Wentzel [31] also observed this type of BEPR behaviour in a number of long-term (> 500 d) lab-scale M/UCT type BNRAS systems at 10 and 20d sludge ages and at 12°C, 20°C and 30°C. Subsequently this type of BEPR has been observed consistently in DEPHANOX [40,41] and ENBNRAS [1–5,42] systems. Therefore, anoxic P uptake and
associated denitrification by PAOs needs to be included in models describing BNRAS systems, particularly the ENBFRAS system.

Attempts have been made to include denitrifying PAOs (DPAOs) into the aerobic P uptake BEPR models by Mino et al. [43], Barker and Dold [9], Murarleitner et al. [16], Filipe and Daigger [44] and Henze et al. [8], amongst others. DPAOs can be included in the models, either by having (1) two different PAO populations, one using oxygen only and the other using oxygen or nitrate, or (2) one PAO population with a reduction factor applied to the aerobic processes for anoxic conditions (as is done with OHO denitrification in ASM1 and 2). Experimental evidence suggests that two PAO populations exist [39]. However, simulations with models based on two PAO populations indicate that in systems that include an aerobic zone invariably the aerobic PAOs will grow to dominance [44]. Recognising this short coming in modelling, the general approach has been to include a single PAO population with different rates and, in some cases, different stoichiometries, for anaerobic and aerobic conditions. The three main models that include anaerobic P uptake BEPR and associated denitrification are:

- **ASM2d** [8],
- the Barker and Dold [9] model,
- the “Delft” model [16].

### 3.1. The ASM2d model

ASM2d is an extension of ASM2 by incorporating two additional anoxic PAO processes, to account for the fact that, under anaerobic conditions the PAOs can use stored PHA to (1) grow and (2) take up P and store it as polyP. The two processes are duplicated from those for aerobic PAO growth and P uptake, respectively, but the aerobic rates are multiplied by a reduction factor, $f_{\text{NO}_3}$, for anaerobic conditions [8].

Anoxic growth of PAOs: The anoxic growth rate of PAOs is obtained by multiplying the aerobic growth rate of PAOs by the reduction factor $f_{\text{NO}_3,G,PAO}$. This accounts for the fact that not all PAOs ($X_{\text{PAO}}$) are capable of anoxic growth (denitrification) and/or the PAOs that denitrify may do this at a reduced rate. The PAO yield coefficient ($Y_{\text{PAO}}$) is the same under both aerobic and anaerobic conditions. However, bioenergetically, the anoxic yield should be reduced compared to the aerobic yield [45], and this reduction has been observed experimentally for OHOs [46].

Anoxic storage of polyP: The anoxic polyP storage rate is obtained by multiplying the aerobic polyP storage rate by the reduction factor $f_{\text{NO}_3,L,PP}$. This accounts for the fact that not all PAOs ($X_{\text{PAO}}$) are capable of anoxic P uptake and/or the PAOs that may do this at a reduced rate. The stoichiometric parameter for P uptake and polyP formation ($Y_{\text{PHA}, \text{polyP}}$) is the same under both aerobic and anoxic conditions, i.e. for the same PHB substrate utilised the same amount of P is taken up and stored as polyP. This is contrary to the observations of Ekama and Wentzel [31] and Hu et al. [47,48], who note that experimentally anoxic P uptake is reduced compared to aerobic P uptake.

It should be mentioned that the reduction factor for OHO anaerobic SBCOD hydrolysis ($f_{\text{NO}_3}$) is increased from 0.1 in ASM2 to 0.4 in ASM2d. This significantly increases the hydrolysis of SBCOD to RBCOD in the anaerobic zone, with the resultant RBCOD available for fermentation to SCFA which are rapidly taken up by the PAOs, and consequently BEPR increases significantly, as discussed above.

### 3.2. The Barker and Dold model

The Barker and Dold [9] model is based on ASM1 [18] for OHOs and ANOs, and the enhanced culture kinetic model [26,27] for PAOs, but includes a number of modifications. The main modifications are: (1) anoxic P uptake and associated denitrification by PAOs and (2) COD loss.

#### 3.2.1. Anoxic P uptake and associated denitrification by PAOs

To include denitrifying PAOs into their model, Barker and Dold [9] accepted that a fraction ($f_P$) of the PAOs can use nitrate as electron acceptor (in the absence of oxygen) for oxidation of the stored PHB and uptake of phosphate. Recognising the short-coming in kinetic modelling of including two competing PAO populations (see above), Barker and Dold [9] included a single PAO population with different kinetics and stoichiometries under aerobic and anaerobic conditions. They duplicated the aerobic PAO growth process (derived from the PAO enhanced culture kinetic model of [27] see above) for anoxic conditions, but multiplied the aerobic PAO rates by the reduction factor ($f_P$, equivalent to $f_{\text{NO}_3,G,PAO}$) for anoxic conditions. This gives a net reduction in the rate of PAO growth and P uptake under anoxic conditions compared with aerobic conditions. Further, recognising that P uptake under anoxic conditions is reduced compared with that under aerobic conditions, the stoichiometric parameter for P uptake per PHB utilised ($f_{\text{P,UPT}}$) was reduced from $f_{\text{T,UPT}} = 0.95 \text{ g P/(g PHB-COD)}$ under aerobic conditions to $f_{\text{T,UPT}} = 0.55 \text{ g P/(g PHB-COD)}$ under anoxic conditions. For aerobic PAO growth, there are four processes in the enhanced culture kinetic model of Wentzel et al. [27] to account for the possibility of ammonia and/or P-limitation. However, in the Barker and Dold model, only one of the aerobic growth process was duplicated for anoxic PAO growth, because Barker and Dold noted that in the anoxic reactor of a continuous-flow BNRAS system,
anoxic PAO growth is likely to occur in the presence of sufficient ammonia and soluble phosphate concentrations. In addition, an anoxic decay process was also included in the Barker and Dold model, duplicated from that under aerobic conditions, but with nitrate instead of oxygen as electron acceptor.

3.2.2. COD loss
A COD loss was included in the Barker and Dold model to account for the frequently observed less than 100% recovery noted in COD mass balances on BNRAS systems (e.g. [4,31,49]). Three COD loss mechanisms are included:

(1) Fermentation process, conversion of F-RBCOD to SCFA by OHOs: This process is modelled with Monod kinetics as in ASM2, but with a yield of $Y_{Y_{H,ANA}}$ for OHOs under anaerobic conditions, which is different from those under anoxic ($Y_{Y_{H,ANOX}}$) and aerobic ($Y_{Y_{H,AER}}$) conditions. A low value of 0.1 mg COD/mg COD is given to $Y_{Y_{H,ANA}}$ and correspondingly, the yield of fermentation product is $(1 - Y_{Y_{H,ANA}})$. Further, in contrast to UCTPHO and ASM2, it is assumed that only a portion ($Y_{ac} = 0.4$) of fermentation products is SCFA ($S_{bsa}$) and the remainder $(1 - Y_{ac} = 0.6)$ is assumed to be lost from the system.

(2) Hydrolysis/solubilisation of SBCOD to F-RBCOD: Two "hydrolysis efficiency factors," $E_{ANOX}$ for anoxic, $E_{ANA}$ for anaerobic have been included to allow for COD loss in this process.

(3) Uptake of SCFA by PAOs: In the model, of the SCFA ($S_{bsa}$) taken up by PAOs, only a fraction ($Y_{PHB} = 0.889$) appears as PHB, with the balance $(1 - Y_{PHB} = 0.111)$ being lost from the system.

3.3. The ‘Delft’ model

Based on the metabolic model for anaerobic/aerobic PAO enhanced cultures with aerobic P uptake only [15], Kuba et al. [11] developed a metabolic model for anaerobic/anoxic PAO enhanced cultures with anoxic P uptake only. Subsequently, Murnleitner et al. [16] integrated these two models to present an integrated metabolic model for anaerobic/anoxic/aerobic PAO enhanced cultures (called the “Delft Model”). In this integrated model, Murnleitner et al. [16] successfully described the two known BEPR behaviours with the same set of kinetic equations and parameters, but with different values for the parameters, i.e. in effect a single PAO population, but with different kinetics and stoichiometries for anoxic and aerobic conditions. The model is based on the biochemistry and stoichiometry of the PAO metabolic pathways, and describes all relevant PAO metabolic reactions with six independent reactions; two for anaerobic metabolism and four for aerobic/anoxic metabolisms.

3.3.1. Anaerobic phase
For the anaerobic PAO stoichiometry, all the internal processes in the anaerobic/anoxic ($A_2$) PAO enhanced culture systems are assumed to be identical to those in the anaerobic/aerobic ($A/O$) PAO enhanced culture systems. For the anaerobic PAO kinetics, Kuba et al. [11] used the same formulations as Smolders et al. [15], but found the maximum specific acetate uptake rate [$q_{ac}^{max}$ mmol/(mmol h)] to be 0.2 for the $A_2$ PAO system, which is lower than the value of 0.4 for the $A/O$ PAO system. In the integrated metabolic model a mean value of 0.3 mmol/(mmol h) is used to be able to describe both systems with one set of kinetic parameters.

3.3.2. Aerobic and anoxic phases
In the integrated model, aerobic and anoxic PAO behaviour is described by six metabolic processes [11]: four processes (polyP formation, PAO growth and maintenance, glycogen formation and PHB degradation) are identical for both the $A/O$ and $A_2$ PAO systems, as described above for the metabolic model of Smolders et al. [15,50,51], because no electron acceptors are involved in these processes. However, the two remaining processes (ATP production and phosphate uptake) depend on the type of electron acceptor:

(1) ATP production from NADH$_2$: (i) with oxygen as electron acceptor, the amount of ATP generated per NADH$_2$ is $\delta_o$ ($\delta_o = 1.8$ [14]); and (ii) with nitrate as electron acceptor, the amount of ATP generated per NADH is $\delta_n$ ($\delta_n = 0.9$ [11]). This implies that PAO energy capture under anoxic conditions is reduced compared with aerobic conditions, in agreement with observations in the literature (e.g. [28,52]).

(2) Phosphate uptake: In the combined model, the amount of phosphate taken up by oxidizing one NADH$_2$ is $\varepsilon$: (i) with oxygen $\varepsilon_o = 7$ [14] and (ii) with nitrate, $\varepsilon_n = 3.5$ [11]. This implies "less efficient" P uptake under anoxic conditions compared with aerobic conditions. Again, this is in agreement with observations in the literature (e.g. [31]). The values for $\varepsilon$ were determined from the relevant $\delta$ values above, via $\varepsilon = 3.8 \delta$.

3.4. Comments on the ASM2d, BIOWIN and “Delft” models
The main objective of this review is to select a model as a basis for further development, thereby to extend application to ENBNRAS systems. From observations on ENBNRAS systems, one aspect of crucial importance is to include anoxic P uptake and associated denitrification by PAOs. Including anoxic P uptake BEPR and associated denitrification by PAOs into the models requires resolution of two main problems:
(a) determination of the concentration of denitrifying PAOs (DPAOs), (b) the reduced P uptake by DPAOs.

3.4.1. The ASM2d model

ASM2d deals with the first problem by multiplying the aerobic PAO growth rate by a reduction factor $\eta_{NO3,G,PAO}$ under anoxic conditions, which can be considered to be equivalent to the proportion of the PAOs capable of denitrification under anoxic conditions. This approach is identical in concept to the manner in which denitrifying OHOs were incorporated in ASM1 and UCTOLD. One difficulty with this approach is to determine the value for $\eta_{NO3,G,PAO}$ for a particular system. ASM2 does not incorporate different aerobic and anoxic yields for either the OHOs or PAOs.

ASM2d does not recognise any differences between aerobic and anoxic PAO metabolism, and therefore would not predict a reduced BEPR when significant anoxic P uptake occurs. In fact, provided the P uptake is not a rate-limited process, all the $\eta_{NO3}$ reduction factor does in this model is to allow P uptake to start earlier under anoxic conditions, but at a reduced rate.

3.4.2. The Barker and Dold model

The Barker and Dold model [9] deals with the first problem by introducing a reduction factor $\eta_p$, which can be viewed as the proportion of the PAOs capable of denitrification under anoxic conditions; this approach is the same as in ASM2d, and has the same difficulties noted above. The model does not include a reduced biomass yield for anoxic PAO growth, but does include a reduced anoxic yield for OHO growth.

With regard to the second problem, reduced anoxic P uptake, the model does recognise differences between aerobic and anaerobic PAO metabolism. In the model, P uptake and polyP formation are stoichiometrically linked to PAO growth/PHB utilisation via the stoichiometric constant $f_{p,upt}$ (P uptake per PHB utilised, g P/g COD). This stoichiometric parameter is given different values for aerobic ($f_{p,upt1} = 0.95$ g P/g COD) and anoxic ($f_{p,upt2} = 0.55$ g P/g COD) PAO growth. This reduces the anoxic uptake by PAOs for polyP formation compared with the aerobic P uptake, in agreement with observations.

3.4.3. The Delft model

In the integrated metabolic model, reduced anoxic P uptake is recognised: Under aerobic conditions, the ATP/NADH$_2$ ratio $\delta_{aerobic} = 1.8$ mol/mol whereas under anoxic conditions $\delta_{anoxic} = 0.9$ mol/mol. The $\delta$ is the only model parameter that differs between the aerobic and anoxic P uptake BEPR. A further effect of the difference in this parameter is reduced anoxic PAO biomass production compared with aerobic production, i.e. in effect a reduced anoxic PAO yield.

From the comparison and discussion above, the Barker and Dold and Delft models both take into account the main two problems for modelling anoxic P uptake and associated denitrification by PAOs in BNRAS systems described above, and thus can serve as a basis for further model development. However, the predictive power of these two models needs to be evaluated against experimental data (see below).

4. Assessment of the Barker and Dold and combined “Delft” models

4.1. The combined Delft model

The “Delft” model was validated and tested by simulation of lab-scale SBR systems operated over a range of sludge ages with oxygen or nitrate as electron acceptor during both start-up and steady state conditions [15,16,50,51,53]). The simulation results indicate that the model is capable of correctly simulating both aerobic and anoxic P uptake BEPR. However, in application of the “Delft” model, it should be noted that (1) all systems were sequencing batch reactor systems, (2) the systems were fed acetate only as influent substrate, i.e. only for P removal, and (3) the systems were P-limited, all of which may influence the BEPR results when the model is applied to multi-reactor, continuous-flow, mixed culture and carbon-limited systems.

To apply the “Delft” model to mixed culture systems receiving real wastewater, Van Veldhuizen et al. [54] combined the Delft model for BEPR with the equations for COD and N conversions of ASM2, following the structure of ASM2 closely. Thus, this model is a hybrid kinetic and metabolic one. The combined model has been validated only in a few full-scale applications [53–55]. Therefore, the predictive power of the combined model needs to be further evaluated. Initially it was attempted to test the combined model by simulating a wide range of experimental data from a number of lab-scale BNRAS systems operated at the University of Cape Town [27,32]. However, it was found that the hybrid model predictions could not be fitted to the experimental data, because the experimental data were obtained based on the UCTPHO kinetic model structure, not the metabolic model structure, i.e. the compounds necessary for the Delft model calibration/evaluation were not measured, e.g. polyP, PHB, glycogen. Furthermore, this combined model does not consider the interactions between the OHOs and denitrifying PAOs, i.e. in mixed culture BNRAS systems receiving real wastewater, the OHOs coexist with the PAOs, which raises the question of how the PAOs will compete with the OHOs for limited nitrate. The kinetics of denitrifying PAOs were derived from the PAO
enhanced culture SBR systems where sufficient nitrate always was supplied to maintain PAO growth [16]. However, experimental observations indicate that the OHOs possibly outcompete the PAOs for nitrate [4], i.e. the DPAOs may experience nitrate-limiting conditions in mixed culture BNRAS systems. In UCT type BNRAS systems, anoxic P uptake only seems to take place when the anoxic reactor is overloaded with nitrate [4, 42, 56].

The developments in the Delft model largely have taken place outside the more universally accepted activated sludge modelling structure, and this indicates caution in following this approach for further model development. However, the Delft models do contain extremely useful information of direct relevance to anoxic PAO growth, with associated anoxic P uptake and denitrification by PAOs, that can be readily incorporated into future models. Of particular interest is the metabolic approach to developing stoichiometric relationships.

4.2. The Barker and Dold model

The Barker and Dold [9] model has not been validated against experimental systems exhibiting significant anoxic P uptake BEPR, so that the measure in which this model would reflect the reduced BEPR with significant anoxic P uptake is not known. Accordingly, the model was evaluated against experimental data, to test its predictive capabilities.

4.2.1. Experimental data for model evaluation

Wentzel et al. [32] reported the results for 30 different lab–scale mixed culture systems. The experimental system configurations were modified Bardenpho, UCT, modified UCT (MUCT) and Johannesburg. The anaerobic zone in these system consisted of either a single, two or four reactors in series, with total mass fraction ranging from 0.09 to 0.5. Recycle ratio to anaerobic reactor was either 0.5, 1 or 2 with respect to the influent flow (for details, refer to [32]).

4.2.2. Simulation results and conclusions

In simulating these systems with the Barker and Dold model, it became evident that a number of processes included in the model require more detailed investigation, in particular, the processes in the anaerobic and anoxic reactors of:

(1) Anaerobic SBCOD hydrolysis to RBCOD,
(2) COD loss mechanisms,
(3) Fraction of PAOs that can denitrify,
(4) Reduced PAO and OHO yields under anoxic conditions.

From simulations, it became evident that the processes (1) and (2) above are highly interactive. Reducing the rate of the one process can be compensated for by changing the rate/stoichiometry of the other. This causes that the Barker and Dold model is very difficult to calibrate against the Wentzel et al. [32] mixed culture data set. Central to the model of Barker and Dold is the COD loss; the degree of this loss has a large impact on the predicted performance. Experimental data in the literature appears to support this COD loss, in that in BNRAS systems some COD is lost that cannot be accounted for in terms of the measured COD mass balance parameters included in this and similar models, e.g. sludge production, oxygen and nitrate utilised [4, 31, 49]. However, the mechanisms whereby this loss occurs are not known. As mentioned above, the COD loss is modelled principally as a reduced yield (40%) of SCFA from F-RBCOD in the anaerobic fermentation process. That the COD loss is associated with an anaerobic process is reasonable since it frequently has been observed in anaerobic/anoxic/aerobic systems (COD mass balances 80–85%), but not in anoxic/aerobic systems (COD mass balances > 90%). However, when this is done it becomes necessary to increase the SCFA concentration obtained by the PAOs because otherwise the BEPR is grossly under-predicted. Hence, the hydrolysis of SBCOD to F-RBCOD in the anaerobic reactor is increased. The net effect of this is a reduced concentration of SBCOD for OHO growth in the system. While this may appear desirable from a design point of view because it leads to reduced oxygen demand, sludge production and reactor volume, none of the kinetic processes to explain COD loss have been validated experimentally. In the simulations it was found that the experimentally determined amount of COD loss appears to vary considerably between different systems and cannot be predicted ab initio. Until greater clarity on this aspect is obtained, it would seem advisable not to include COD loss in a BNRAS system kinetic model, as it may lead to an incorrect assessment of the system behaviour. Clearly, the COD loss does require further investigation.

With regard to (3), it was found that the fraction of PAOs that can denitrify (DPAOs) varied considerably from system to system. While it appeared that this behaviour is stimulated in systems with large anoxic mass fractions (40–55%) overloaded with nitrate [4, 42, 56], casual links for the variation in the magnitude of anoxic P uptake could not be established, and hence the DPAO fraction could not be predicted, but had to be calibrated in model application. This is undesirable, since in the Barker and Dold model anoxic P uptake is reduced compared to aerobic P uptake, so that the value for the fraction of DPAOs has a significant impact on the predicted P removal.

With regard to (4) reduced PAO and OHO yields under anoxic conditions, in the model of Barker and Dold a reduced anoxic OHO yield is accepted, but not a reduced PAO yield. This inconsistency requires
resolution. (This has been addressed in more recent implementations of the model in the computer programme BIOWIN.)

5. Closure

The intention in this paper was to review and evaluate kinetic simulation models for BNRS systems, to select the most suitable to serve as a starting point for extension to include ENBNRAS system application. Initially it was thought that the best approach would be to select the most complete model available, and to modify and calibrate this model. From the literature, the model of Barker and Dold [9] appeared to fit this criterion. However, from an assessment of, and simulations with this model it became apparent that the model was inadequately validated, and that the highly interactive nature of the processes (particularly with COD loss) makes calibration difficult; a large number of sets of constants can be obtained that give the same net behaviour. Accordingly, it was decided to revert to the most extensively validated model, namely UCTPHO, and extend this model in stages to incorporate those processes omitted in the model. In particular, requiring inclusion is the anoxic behaviour of PAOs, i.e. anoxic growth of PAOs with associated anoxic P uptake and denitrification. Inclusion of anoxic PAO behaviour must take due cognisance of reduced PAO P uptake and polyP formation under anoxic conditions, reduced PAO anoxic yields, and the relative reduction in the rates of the PAO mediated processes, all compared with aerobic conditions. Fortunately, in the literature and existing models there is a substantial body of information of direct relevance on these aspects. Other important aspects identified to require consideration are anaerobic SBCHD hydrolysis and COD loss. Both processes have significant impact on the simulated performance. The development of the extended model will be presented and evaluated in a series of future papers.

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