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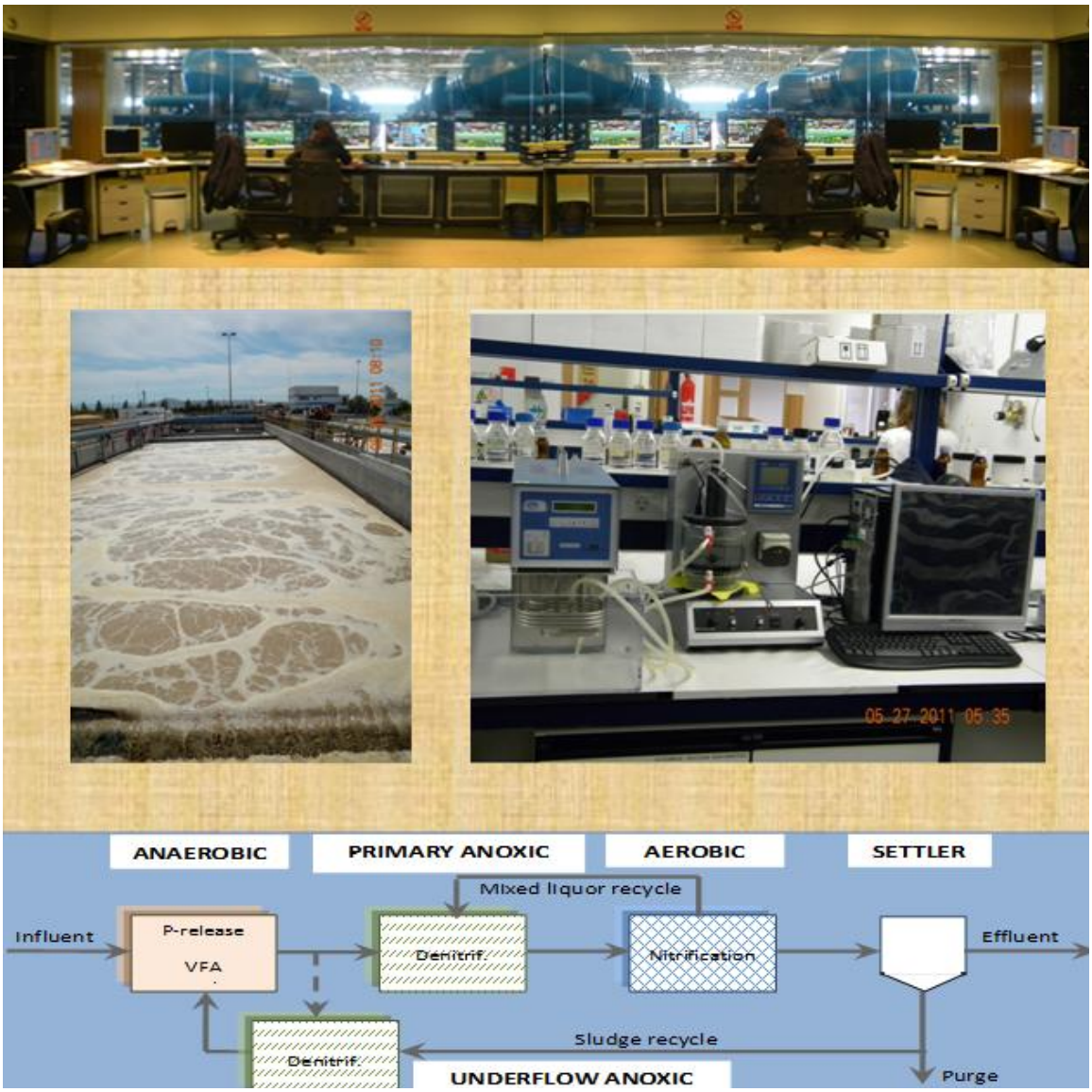

An Integrated Model to describe microbial populations in Enhanced Biological Phosphorus Removal (EBPR) systems.

Benly Liliana Ramírez Higareda

MSc Thesis (MWI-SE)
April, 2012

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An Integrated Model to describe microbial populations in Enhanced Biological Phosphorus Removal (EBPR) systems.

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The findings, interpretations and conclusions expressed in this study do neither necessarily reflect the views of the UNESCO-IHE Institute for Water Education, nor of the individual members of the MSc committee, nor of their respective employers.

To my family, friends and Jorge

Abstract

This research proposes an integrated mathematical model that evaluates the occurrence of relevant microbial communities present in full-scale Enhanced Biological Phosphorus Removal (EBPR) systems, which were studied recently and have not been incorporated yet in an integrated model.

In order to develop the integrated mathematical model, four microbial communities included in a recently developed metabolic model for EBPR systems (Oehmen *et al.*, 2010) were incorporated in the matrix of the ASM2d+TUD model (Meijer, 2004). The proposed model in this thesis was called the 'Extended ASM2d+TUD model'.

The impact on the model parameters when the pH and influent composition fluctuates within a typical range for municipal wastewater was measured through a sensitivity analysis. Based on the results, it was proposed an approach to consider the effect of the latter variables in the biological P removal in a simple yet reliable manner. Additionally, the proposed model included multistep nitrification/denitrification processes that may help to quantify the accumulation of undesirable intermediates.

The software AQUASIM (a simulator for aquatic environments) was used to evaluate the performance of two microbial communities using the proposed model, without any change in the original stoichiometric and rate values. The results were compared with laboratory data. Furthermore, this system was simulated when the pH, temperature and influent composition change. The results suggest that the part of the model that was tested is reliable, and the same approach can be used to test the remaining components of the model before a further calibration and validation of the model.

It is expected that this model will help to evaluate population dynamics and gain a better understanding of their interactions, as well to predict the operating conditions that enrich or restrict certain microbial populations. This can potentially help to design and optimize the operation of wastewater treatment plants with EBPR systems, especially those that experience unexpected low P-removal efficiencies.

Keywords: Enhanced biological phosphorus removal (EBPR); Phosphorus accumulating organisms (PAO); Denitrifying phosphorus accumulating organisms (DPAO); Glycogen accumulating organisms (GAO); Modelling; ASM2d+TUD model

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But this studies were not only an academic experience but also a personal adventure surrounded by people that I met in Delft and I will take back home inside my heart. Thank you very much to all my friends here for the great moments we lived in this piece of land and time that fortunately, we enjoyed together.

Also, I want to thank the people that I love in Mexico. Thanks to my family, especially my parents who have always supported and trusted in me, and with whom I have shared this dream since it was just a platonic idea. Thanks to my friends, whom I can always trust and share my heart dressed with prose. And last but not the least, thanks to Jorge, the man that I love, my best friend, and the one with whom I want to share my future.

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List of Abbreviations

A2O	3-stage Modified Bardenpho
ANO	Autotrophic nitrifying organisms (NH_4^+ to NO_3^-)
AOO	Ammonia oxidizing organisms (NH_4^+ to NO_2^-)
ASM2d+TUD	Activated Sludge Model 2d modified later by Delft University of Technology
BCFS	Biological-chemical phosphorus and nitrogen removal
BG	Non-Denitrifying Competibacter (GAO)
COD	Chemical oxygen demand
DBG	Denitrifying Competibacter (GAO)
DDEF	Denitrifying *Defluviicoccus* (GAO)
DEF	Non-denitrifying *Defluviicoccus* (GAO)
DPAO	Denitrifying Polyphosphate-accumulating Organisms
EBPR	Enhanced biological phosphorus removal
GAO	Glycogen-accumulating organisms
JHB	Johannesburg
MCA	Multicriteria Analysis
MJHB-IHE	Modified Johanesburg
MUCT	Modified UCT
N	Nitrogen
N_2	Nitrogen gas
N_2O	Nitrous oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NOO	Nitrite oxidizing organisms (NO_2^- to NO_3^-)
NRMSD	Normalised root mean squared deviation
OHO	Ordinary Heterotrophic Organisms
P	Phosphorus
PAO	Phosphorus-accumulating Organisms
PAOI	*Accumulibacter* Type I (PAO)
PAOII	*Accumulibacter* Type II (PAO)
PHA	Poly-β-hydroxyalkanoates
PO_4	Orthophosphate (phosphates)
PP	Poly-phosphate
VFA	Volatile fatty acids
WWTP	Wastewater treatment plant

1 Introduction

1.1 Background

Phosphorus (P) is one of the elements required in every living cell, from which it is possible to synthesize essential macromolecules that compose life, for instance: DNA, RNA, ATP and phospholipids. Thus, it is not surprising that P represents a growth limiting factor and an appropriate balance of this element in the nature is required. Nevertheless, all over the world it is common to find this element in excess or scarcity, causing environmental, social and economical problems. For example, the excess of P in the discharges to surface water bodies is the main reason of eutrophication, which changes the nature ecosystem and makes highly difficult to treat the water for drinking purposes. On the other hand, scarcity of mineral P to use it as a fertilizer is causing a price increase of this non-renewable element, in a world of growing food demand.

Based on the reasons mentioned above, eutrophication is the one of the main driving forces for P removal research, and fertilizer crisis is the key factor to think about P recovery.

There are biological and chemical processes that can be used to remove P from wastewater. Nevertheless, generally it is more recommendable to apply biological processes, when the wastewater characteristics and site conditions allows to do so. The main advantages of biological removal processes is that they have lower operational costs because no chemical addition is needed and lesser sludge is produced compared to chemical technologies.

Additionally, biological sludge is more environmental friendly than chemical sludge, which can be recycled through land application when the concentrations of pathogens and heavy metals in the biological sludge are below the recommended (or restricted) limits (Wang *et al.*, 2004). This means that biological processes allow not only removing P from the wastewater but also recovering and reusing it.

As a result, the biological process has been studied and applied increasingly since the late 1950s, when it was noticed that some activated sludge systems that had an anaerobic step before the main biological process were able to remove more P than required for the anabolic purposes of the heterotrophic biomass. After some research, it was found that the process configuration of these wastewater treatment plants (WWTPs) promoted the growth of microorganisms with higher phosphorus intake than ordinary heterotrophs (OHO), known as Phosphorus Accumulating Organisms (PAOs), due to the intracellular accumulation of polyphosphates (PP).

The systems that promote the PAOs growth are widely called Enhanced Biological Phosphorus Removal (EBPR) processes.

P removal is promoted when there is proliferation of PAO communities because they are able to store in their cells approximately 0.38mgP/mgVSS, which is about twenty times higher than the assimilation of P required by Ordinary Heterotrophic Organisms (OHOs) for their anabolic processes. In a well designed EBPR configuration, PAOs can grow and comprise up to 40% of the total active organisms present in an activated sludge system (Henze *et al.*, 2008).

One of the main challenges to apply EBPR configurations at full-scale is the complexity of the biochemical mechanisms involved and the interrelations among microbial ecosystems. These interactions can be described by using mathematical models, in which the relevant components, variables and processes are organized in a framework that helps to represent certain system as simple and reliable as possible (Henze *et al.*, 2008).

Modelling is increasingly used to optimize and upgrade existing WWTPs, also to select control strategies under different scenarios, and to evaluate new designs (Henze *et al.*, 2008). In many cases, modelling has helped to save time and money in the mentioned decision making processes. As a result, it is becoming a powerful tool to understand, simulate and predict the performance of WWTPs.

1.2 Problem statement

There are some cases of full-scale EBPR plants showing failure or presenting lower P removal efficiency than was expected during the design (Blackall *et al.*, 2002, Hartley & Sickerdick, 1994, Stephens *et al.*, 2004, Thomas *et al.*, 2003).

Some studies report that P removal tends to become more unstable under anaerobic-anoxic-aerobic conditions compared with only anaerobic-aerobic systems (Saito *et al.*, 2004). Nevertheless, due to effluent quality requiremente, EBPR systems are almost always combined with nitrogen removal in full-scale plants, in which anaerobic/anoxic/aerobic environments are present.

Recent research has found that in addition of PAOs, there are other communities of microorganisms that exist in EBPR systems and have a strong impact on the process performance (Burow *et al.*, 2007, Crocetti *et al.*, 2002, Gu *et al.*, 2005, Kong *et al.*, 2002, 2006, Thomas *et al.*, 2003, Wong *et al.*, 2005).

For instance, Glycogen Accumulating Organisms (GAOs) also take up volatile fatty acids (VFAs) anaerobically but do not remove P. Certain reports speculate that the proliferation of GAOs can lead to nitrite accumulation, which inhibit some processes in PAOs (Saito *et al.*, 2004) and is linked to nitrous oxide (N_2O) emissions (Zeng, Yuan, *et al.*, 2003, Zhou *et al.*, 2008), which is not desired due to its significant contribution to the greenhouse effect (Kramlich & Linak, 1994).

Despite the disadvantages mentioned before, when the system performance is efficient, EBPR configurations coupled with N removal present important advantages. Recent studies have found that a microbial community of denitrifying PAOs is able to remove P under anoxic conditions, using nitrate as the electron acceptor. This suggests that simultaneous N and P removal is economically attractive in EBPR configurations because the demand for limiting carbon sources, oxygen requirements and sludge production can be reduced (Kuba *et al*., 1994a, 1996; Oehmen *et al.*, 2007).

The combination of process configuration, operating parameters and environmental conditions defines the occurrence of microbial populations and the P removal efficiency. The enhancement or restriction of the growth of certain populations like GAOs or denitrifying PAOs is a key factor to optimize an EBPR system (Oehmen *et al.*, 2010)**.**

In order to be able to assess in a cost effectiv way the influence of microbial populations that impact in EBPR performance, an extension of currently used integrated models is needed. It may help to evaluate population dynamics, have a better understanding about their interactions and predict the operating conditions that enrich certain populations. Unfortunately, the last studies regarding the competition and co-existence of other microbial communities with PAOs is not yet in one complete model which considers P, N and organic matter removal.

2 Literature review

2.1 Biological Phosphorus removal mechanism

Phosphorus Accumulating Organisms (PAOs)

In an activated sludge system, PAO's growth can be promoted if the biomass passes through alternating anaerobic and anoxic/aerobic phases (see Figure 1). During the anaerobic stage, PAOs have a competitive advantage over Ordinary Heterotrophic Organisms (OHOs) to assimilate carbon source. PAOs are able to take up volatile fatty acids (VFAs) and store them as Poly-β-hydroxyalkanoates (PHAs), whereas OHOs only can ferment the substrate without consuming it.

The energy required for the storage of VFAs as PHAs in PAOs comes from the intracellular consumption of polyphosphates (PP) and glycogen, which leads to the consequent release of ortophosphates (Oehmen *et al.*, 2007).

Afterwards, when PAOs are in an anoxic or aerobic environment, they utilize PHA as a carbon source. In this step, PAOs take up P to synthesize PP again and also to generate glycogen and new biomass. For this reason, PAOs growth is directly related with P removal (Henze *et al.*, 2008) and so forth the EBPR systems must be designed to enhance PAO proliferation.

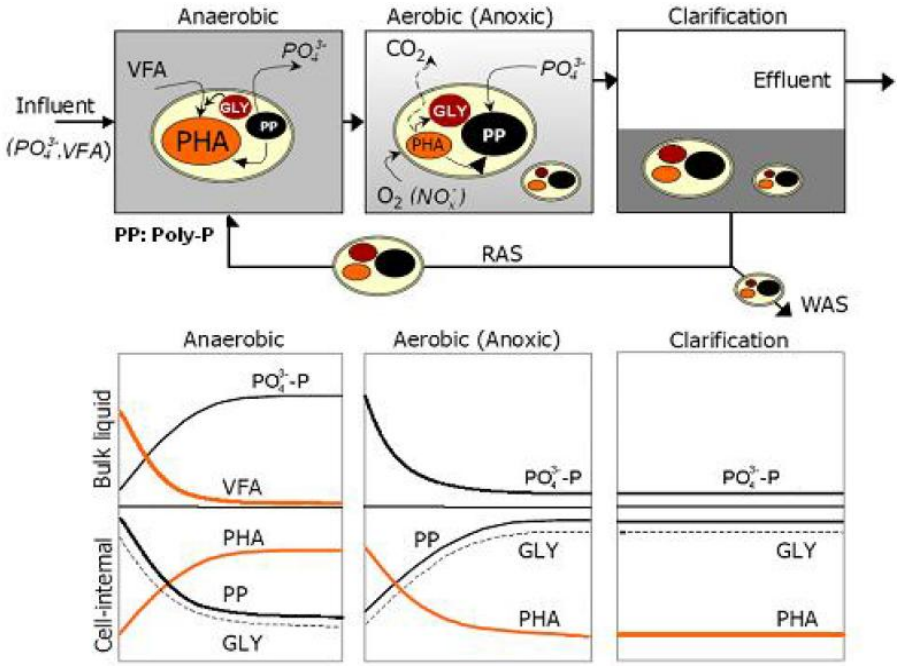


Figure 1: Schematic representation of the PAOs activity in a EBPR plant (Lopez-Vazquez, 2009)

P removal coupled with denitrification

PAOs are able to remove P under anoxic conditions, using nitrate as the electron acceptor. By applying molecular biology tools, Ahn *et al* (2002) confirmed that the population of microorganisms involved in P removal process changes depending on the electron acceptor used (oxygen, nitrate). PAOs can degrade PHA to take up P either under aerobic or under anoxic conditions, but the ATP production depends on the electron acceptor; which is about 40% lower under anoxic than under aerobic conditions (Kuba *et al.*, 1996b, Smolders *et al.*, 1994).

Competitors of PAOs

Despite that the alternating anaerobic-aerobic conditions stimulate the proliferation of PAO, a combination of certain factors such as high concentration of acetate in VFAs influent, low pH levels or relatively high temperatures can promote the occurrence of other microbial communities, such as GAOs, that can compete with PAOs (Brdjanovic *et al.*, 2000, Ky *et al.*, 2001, Lopez-Vazquez *et al.*, 2009). They also take up the limiting VFAs anaerobically, but do not remove P during the aerobic/anoxic phase because their source of energy is only glycogen (see Figure 2).

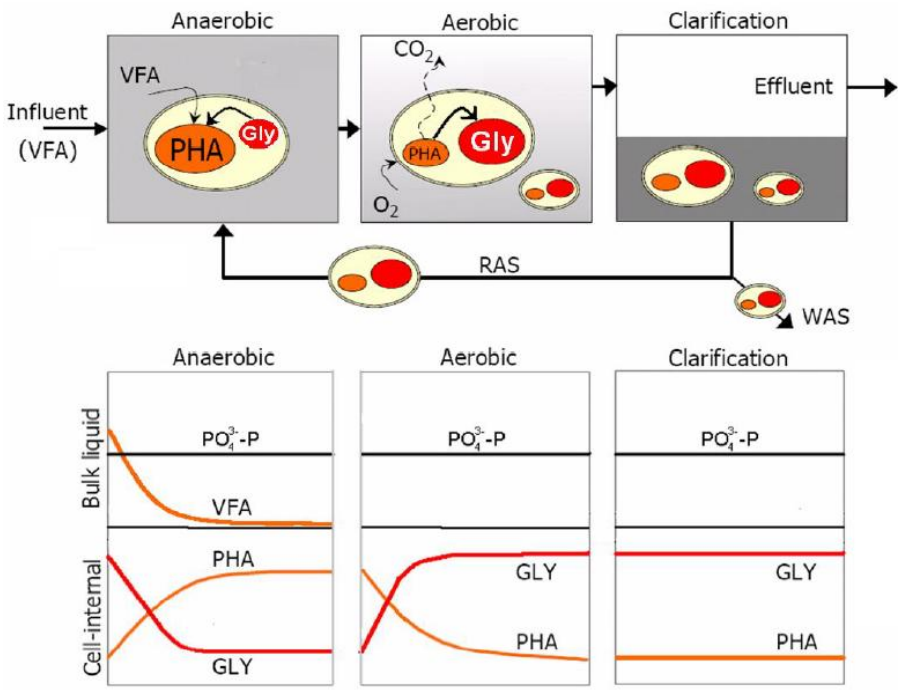


Figure 2: Schematic representation of the GAOs activity in a EBPR plant (Lopez-Vazquez, 2009)

2.3 EBPR full scale configurations

P removal at full scale can be done in the mainstream or in a sidestream process. Side stream processes have lower PAO fractions because not all the mixed liquor is exposed to anaerobic conditions (López-Vázquez *et al.*, 2008), and usually a chemical addition to recover P by precipitation is needed, like in the PhoStrip process (Levin & Shapiro, 1965).

The most common EBPR configurations used for mainstreams treatment than includes COD, N and P removal without any chemical addition are:

a) 3-stage Modified Bardenpho
b) Johannesburg (JHB)
c) Modified UCT (MUCT)

Also, it is possible to combine biological P removal with chemical precipitation, like the Biological-chemical phosphorus and nitrogen removal (BCFS, Barat & van Loosdrecht, 2006). Nevertheless, the addition of chemicals can reduce the availability of phosphorus for PAO growth if there is not an adequate P-effluent set point (Janssen *et al.*, 2002).

2.4 Modelling EBPR

There can be infinite combinations of process configurations, site conditions and wastewater characteristics. For this reason, modelling becomes a powerful and flexible tool to test the feasibility of certain configuration or process control.

In order to simulate EBPR systems, several models have been developed, like: Barker & Dold (Barker & Dold, 1997), ASM2d (Henze, 2000), ASM3+BioP (Rieger *et al.*, 2001), UCTPHO+ (Hu *et al.*, 2007) and ASM2d+TUD (Meijer, 2004).

A complete revision of these models and an analysis of their limitations was done by Hauduc *et al* (2010), who explained that ASM2d and ASM2d+TUD models do no neglect fermentation hydrolysis in the anaerobic reactor but, on the other hand, do not consider that alkalinity can be a limiting rate factor for some processes. For this reason, these two models are suitable when fermentation play an important role in the processes, and also when alkalinity is in excess or it can be assumed that there are control measures in the system to maintain alkalinity above certain level.

ASM2d has been used and modified for specific purposes. For instance, some versions of ASM2 have incorporated more parameters to the model, like glycogen storage, GAOs activity (Manga *et al.*, 2001, Mino *et al.*, 1995), prediction of pH (Serralta *et al.*, 2004), propionate use (Pijuan *et al.*, 2004) and competition between PAOs and non-polyphosphate accumulating heterotrophs (Schuler, 2005).

2.4.1 The ASM2d+TUD model

One of these modifications was the ASM2d+TUD model (Meijer, 2004), in which the metabolic and ASM2d models were combined in order to model P and N removal at full scale WWTP in configurations like UCT, modified UCT and A2N (Brdjanovic *et al.*, 2000, Hao *et al.*, 2001, Meijer *et al.*, 2001, van Veldhuizen *et al.*, 1999).

The ASM2d+TUD model includes three microbial populations: (1) the Ordinary Heterotrophic Organisms (OHOs) that are the main organisms responsible for organic matter removal and denitrification, (2) the Autotrophic nitrifying organisms (ANOs) that carry out nitrification assuming 1-step process ($NH_4^+ \rightarrow NO_x^-$), and (3) the Phosphorus-accumulating Organisms (PAOs) that remove P due to their high capacity to store intracellular PP.

Regarding P-removal processes, the assumption of the ASM2d+TUD model is that PAO is the only community involved in EBPR processes, considering an aerobic P-removal rate and a reduction factor (η_{qPAO}) for its activity under anoxic conditions. The P removal coupled with

denitrification is assumed to be a 1-step process ($NO_x^- \rightarrow N_2$), and the source of VFA is considered as only acetate. The impact of temperature in the process performance is incorporated in the model.

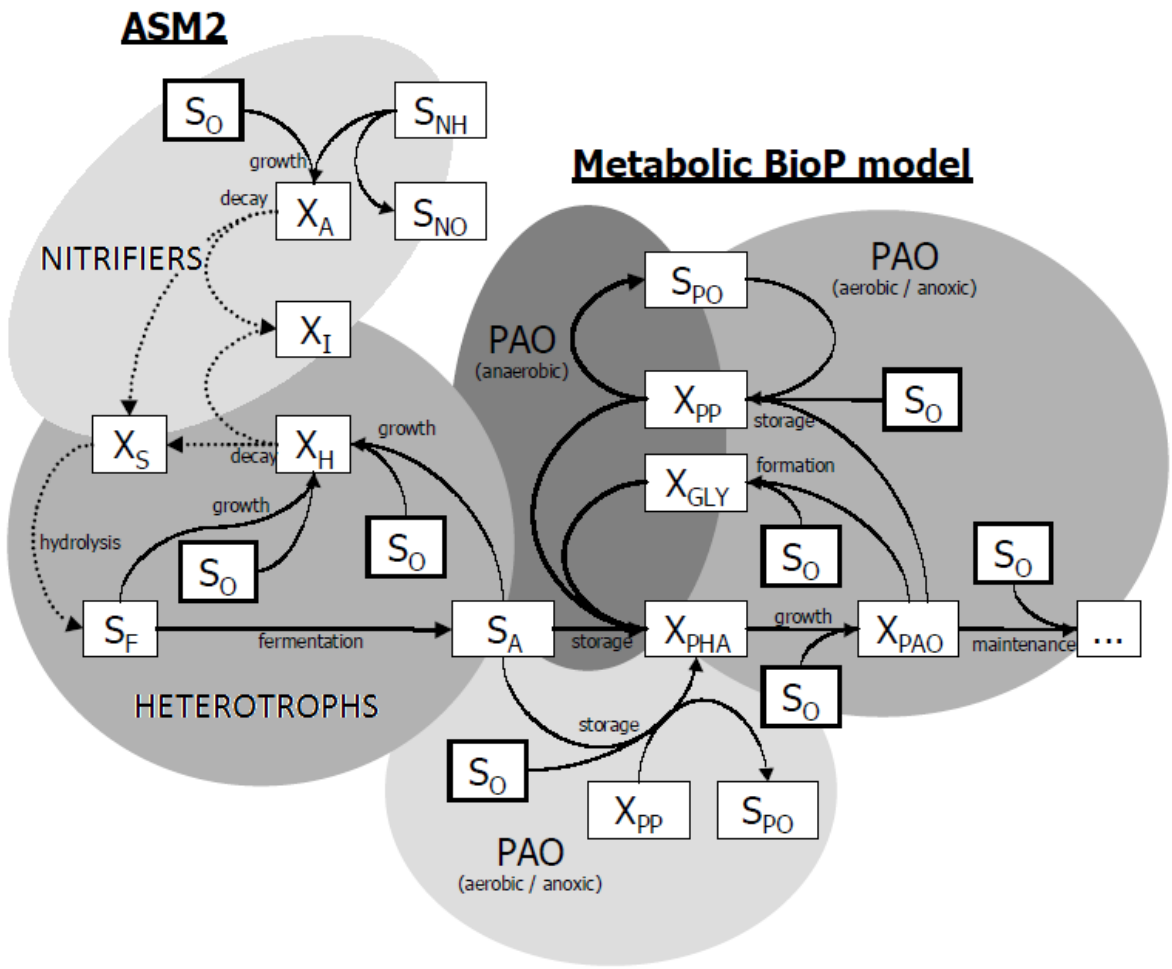


Figure 3: The interactions in the ASM2d+TUD (Meijer, 2004)

2.4.2 The Metabolic model

However, as mentioned earlier, recent studies have shown that PAO is not a homogenous group of microorganisms, it can be divided into subgroups with different metabolic capacities where the role of electron acceptors (such as nitrate and nitrite) appears to play a major role on the abundance and occurrence of the different PAO sub-groups. Moreover, there are other microorganisms that can compete with PAO affecting the P removal efficiency (Oehmen *et al.*, 2010).

Consequently, Oehmen *et al*. (2010) proposed a Metabolic model to describe six groups of microorganisms involved specifically in P removal processes with denitrifying capabilities. The classification of these microbial populations shown in Table 1 depended on the species

(*Accumulibacter,* Competibacter, *Defluviicoccus*) and also on the capacity to reduce nitrate (NO_3), nitrite (NO_2) and nitrous oxide (N_2O):

Table 1. Anoxic metabolism of microbial populations involved in EBPR systems (Oehmen *et al.*, 2010)

	Abbr.	Microbial population	Metabolic characteristics
1	PAO I	*Accumulibacter* Type I (PAO)	$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$
2	PAO II	*Accumulibacter* Type II (PAO)	$NO_3^- \not\rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$
3	DBG	Denitrifying Competibacter (GAO)	$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$
4	BG	Non-Denitrifying Competibacter (GAO)	$NO_3^- \not\rightarrow NO_2^- \not\rightarrow N_2O \not\rightarrow N_2$
5	DDEF	Denitrifying *Defluviicoccus* (GAO)	$NO_3^- \rightarrow NO_2^- \not\rightarrow N_2O \not\rightarrow N_2$
6	DEF	Non-denitrifying *Defluviicoccus* (GAO)	$NO_3^- \not\rightarrow NO_2^- \not\rightarrow N_2O \not\rightarrow N_2$
✕ Process that the microorganism cannot do.			

These communities were described in the Metabolic model (Oehmen *et al.*, 2010) assuming 3-step denitrification process and a VFA composed by acetate and propionate. This model considers the impact of pH on the kinetics and stoichiometry of the processes.

2.5 Anoxic metabolism of microbial populations

The nitrification is done by two autotrophic microorganisms: *Nitrosomonas* and *Nitrobacter* (Koops *et al.*, 1991). The ammonia oxidizers (AOO) or genus *Nitrosomonas* oxidize NH_4 to NO_2 in two steps ($NH_4 \rightarrow NH_2OH \rightarrow NO_2$) and the nitrite oxidizers (NOO) or genus *Nitrobacter* oxidize NO_2 to NO_3(Colliver & Stephenson, 2000).

On the other hand, denitrification can be asumed as a 4-step process, when the most oxidized form (nitrate) and the intermediary nitric oxide (NO) are taken into account as shown in Figure 4. Nevertheless, most of the time NO is neglected for domestic waste water treatment plants because its concentration is very low and does not affect the denitrification processes.

In nature, the N_2O and NO are mainly produced in agriculture and forest lands and in aquatic ecosystems (Kramlich & Linak, 1994). The anthropogenic production of those gases is mainly found in explosive manufacturing, fertilized soils, cellophane and metal production and nuclear weapon production (Ghafari *et al.*, 2008, Watanabe *et al.*, 2001).

In biological reactor are associated to nitrification and denitrifcation processes (Colliver & Stephenson, 2000). During nitrification is associated with low concentration of oxygen, short retention times, low pH and low ratio of carbon/nitroben in the aerobic tank (Hanaki *et al.*, 2011, Hwang *et al.*, 2006, Schulthess & Gujer, 1996, Schulthess *et al.*, 1994, Thörn & Sörensson, 1996).

The release of nitrification/denitrification intermediates are not desired in the environment due to their toxic or polluting attributes (Schulthess & Gujer, 1996). N_2O has a lifetime of 130 years aproximately and is 10-100 times and 100-1000 times more greenhouse effect thatn methane and carbon dioxide respectively. On the other hand, NO cause depletion of ozone layer (Kramlich & Linak, 1994).

Alinsafi *et al* .(2008) suggests that NO_2 accumulations causes N2O emission due to the inhibitory effect of NO_2 in N_2O reduction.

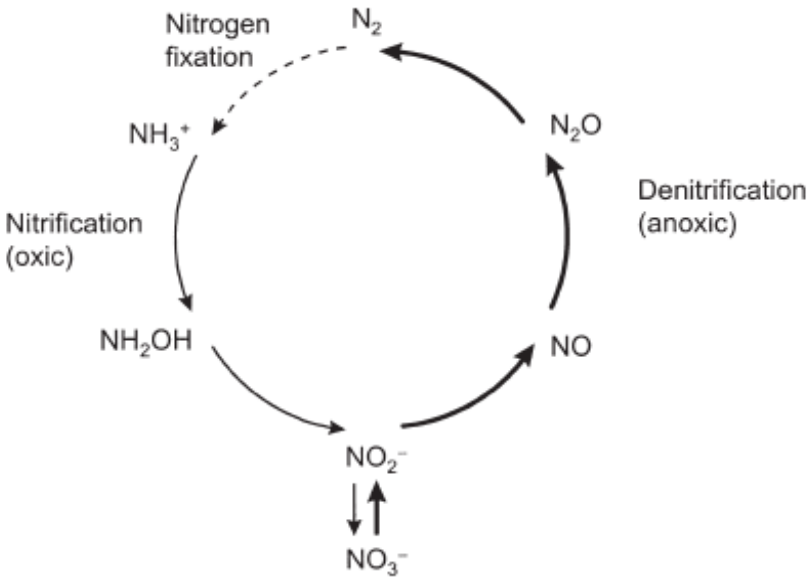


Figure 4: The nitrogen cycle. Thin -lined arrows, nitrification; thick-lined arrows, denitrification; dashed arros, nitrogen fixation. (Colliver & Stephenson, 2000)

3 Methodology

3.1 Objectives

General objective

To build a more complete integrated mathematical model that evaluates the occurrence of relevant microbial communities present in full-scale EBPR systems considering the impact of different environmental and influent conditions.

Specific objectives

In order to achieve the main objective of this thesis, the work was divided into three different phases with specific objectives:

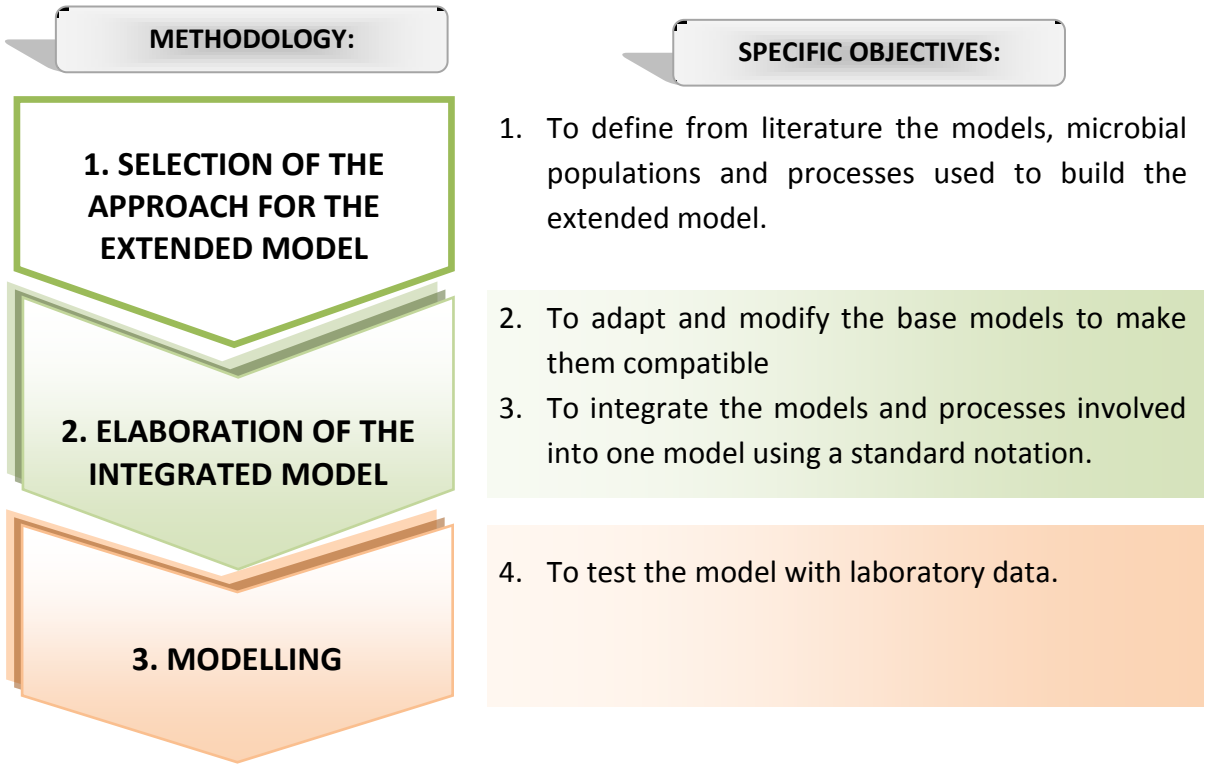


Figure 5: Methodology and objectives of the thesis

3.2 Selection of the approach for the Extended model

Model

For this study, the model ASM2d+TUD (Meijer, 2004) was used as a base model because it is one of the most complete approaches and does not neglect some biological processes such as the fermentation processes.

In order to develop the integrated mathematical model, the matrix of the ASM2d+TUD model (Meijer, 2004) was extended with some communities included in the Metabolic model (Oehmen *et al.*, 2010). For this reason, the model proposed in this thesis was called the 'Extended ASM2d+TUD model'.

Microbial populations

As a result of the integration of both models, seven microbial populations were involved:

Table 2: Microbial populations in the integrated model ASM2d+TUD+IHE

No.	*Abbr.*	*Microorganism*	*Type of process*	*Model*
1	**OHO**	Ordinary Heterotrophic Organisms	**COD removal**	**ASM2d+TUD**
2	**AOO**	Ammonia oxidizing organisms	Nitrification	Adaptation of ASM2d+TUD
3	**NOO**	Nitrite oxidizing organisms		
4	**PAOI**	*Accumulibacter* Type I (PAO)	P removal	Metabolic model
5	**PAOII**	*Accumulibacter* Type II (PAO)		
6	**GB**	Non-Denitrifying Competibacter (GAO)	Competition with P removal processes	Metabolic model
7	**DGB**	Denitrifying Competibacter (GAO)		

3.3 Elaboration of the integrated model

The models selected to build the integrated model used different languages and components. Moreover, some processes were duplicated, some were not in the scope of this thesis, and others did not consider variables that in an integrated scenario would have an impact. Hence, both models required important modifications before their integration in a single model.

3.3.1 Modifications of the ASM2d+TUD Model (Meijer, 2004)

The modifications described below for the ASM2d+TUD model in order to build the Extended ASM2d+TUD model (proposed in this thesis) are shown schematically in Figure 6.

Exclusion of the processes of PAO

From the microorganisms included in the ASM2d+TUD model (OHOs, ANOs and PAOs), PAOs were replaced by PAOI and PAOII subgroups.

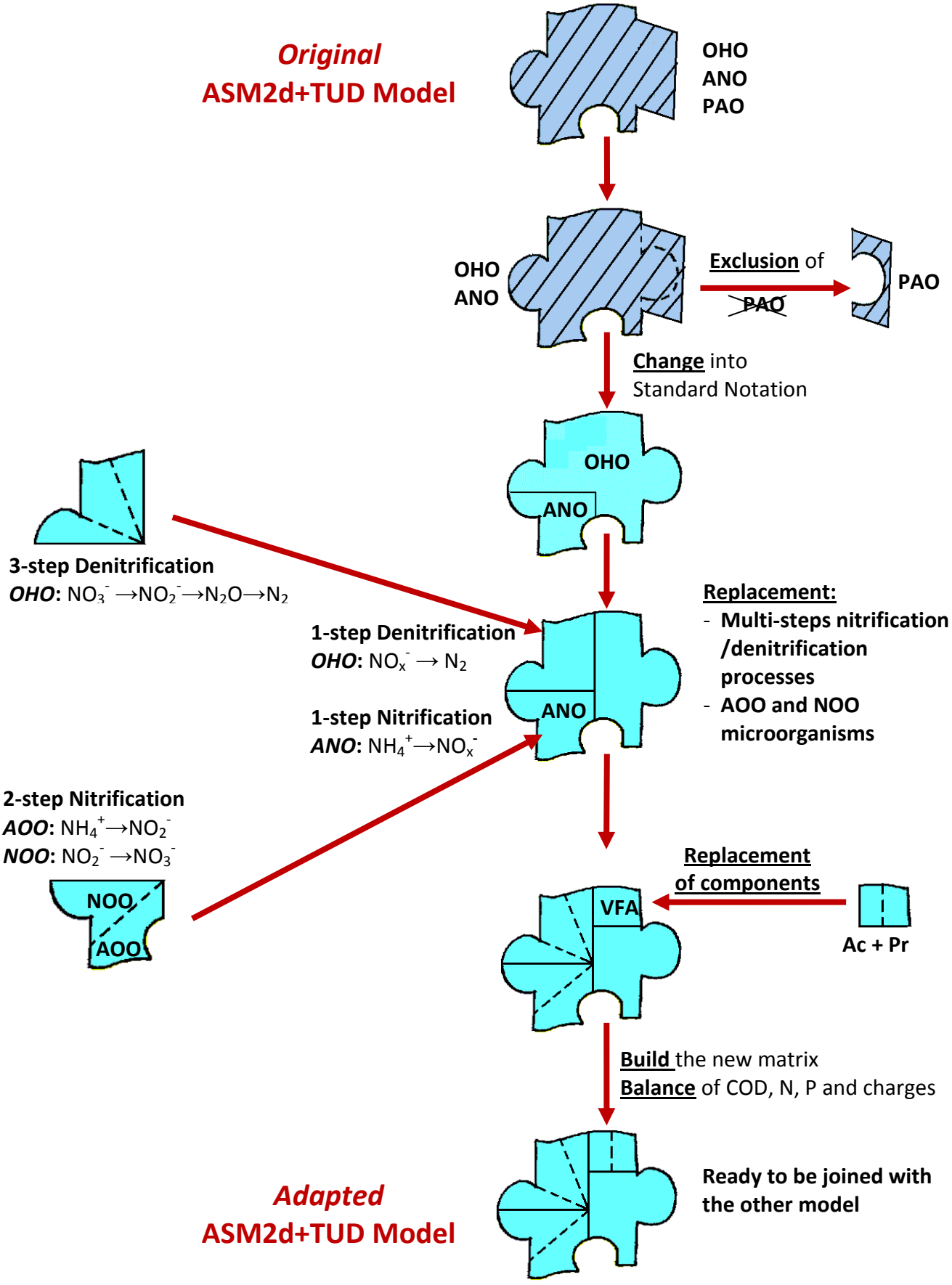


Figure 6: Adaptation of ASM2d+TUD Model

Use of standard notation

Recently, Hauduc (2010) reviewed and corrected different typing errors and inconsistencies found in the ASM2d+TUD model. In the same publication, the nomenclature of the model was updated using the standard notation proposed by Corominas et al. (2010). The corrections and updates made to the ASM2d+TUD model were considered to build the integrated model.

Addition of components

The use of the same components in both, the ASM2d+TUD model and the Metabolic model (Oehmen *et al.*, 2010)**,** was indispensable in order to combine them afterwards. For that reason, the VFA component (assumed as only acetate in the ASM2d+TUD model) was split into acetate and propionate, as considered in the Metabolic model (Oehmen *et al.*, 2010)**.**

Additionally, it was necessary to replace the global nitrate/nitrite (NOx) assumed in the ASM2d+TUD model by the three components (NO_3, NO_2 and N_2O) included in the Metabolic model (Oehmen *et al.*, 2010). In order to do so, multi-steps nitrification/denitrificacion processes were incorporated.

Addition of multi-steps nitrification /denitrification processes

The denitrification process in the ASM2d+TUD model was extended from 1-step process ($NO_x^- \rightarrow N_2$) to 3-step process ($NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$), as considered in the Metabolic model (A. Oehmen *et al.*, 2010). In order to include the intermediaries produced in the aerobic tank, it was also necessary to extend in the ASM+TUD model the 1-step nitrification ($NH_x \rightarrow NO_x^-$) to a 2-step nitrification ($NH_x \rightarrow NO_2^- \rightarrow NO_3^-$).

Mass and charges balance

Once the ASM2d+TUD model was modified, the stoichiometry was ordered into a new matrix. In order to make the mass and charges balance, the contribution of each component in terms of chemical oxygen demand (COD), nitrogen (N), phosphorus (P) and charges was calculated. The components produced and consumed in each processes were multiplied by their COD, N, P and charge

Finally, once it was probed that the mass and charges balances did close, the stoichiometry was considered consistent. Therefore, the ASM2d+TUD model adapted in this thesis was ready to be joined with the Metabolic model (Oehmen *et al.*, 2010)**.**

3.3.2 Modifications of the Metabolic Model (Oehmen *et al.*, 2010).

The modifications described below for the metabolic model in order to build the Extended ASM2d+TUD model (proposed in this thesis) are shown schematically in Figure 7.

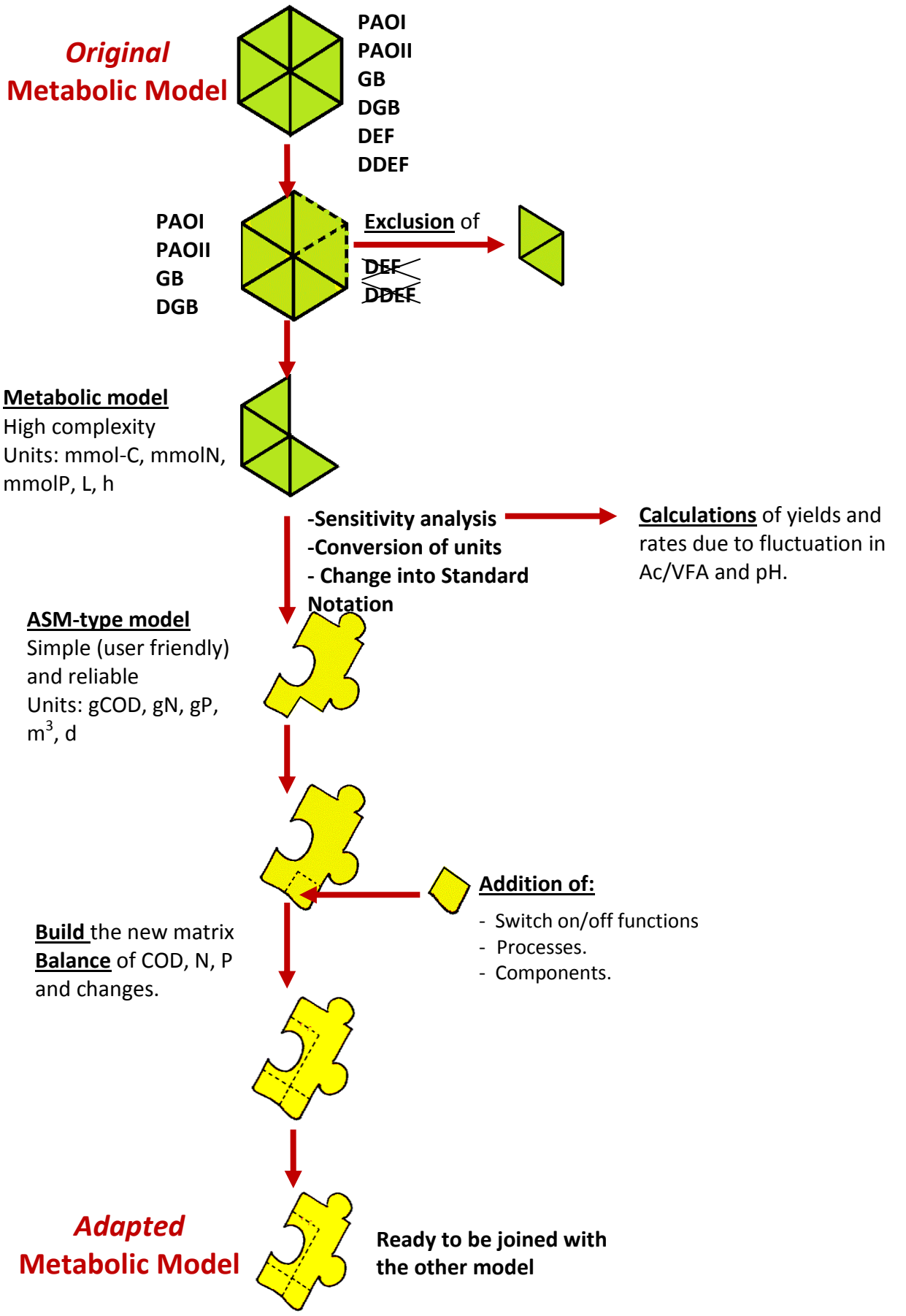


Figure 7: Adaptation of the Metabolic model

Exclusion of DEF and DDEF processes

From the classification proposed by Oehmen *et al*. (2010), the two sub-groups of Phosphorus-accumulating Organisms (PAOI and PAOII) and the two sub-groups of *Competibacter* (BG and DBG) were integrated into the Extended ASM2d+TUD model (proposed in this thesis). However, in this study it was decided to neglect the communities corresponding to *Defluviicoccus* (DDEF and DEF) due to their low presence in most of the municipal wastewaters (López-Vázquez *et al.*, 2008). As a consequence, all the processes and components related with these last two communities were excluded from the Metabolic model (Oehmen *et al.*, 2010)**,** adapted in this thesis.

Sensitivity analysis

A sensitivity analysis consisted in measuring the impact on the stoichiometric and kinetic parameters when the pH and quality influent fluctuates within a range that is characteristic of municipal wastewater. The main purpose of this analysis was to reduce the complexity of the model due to several interrelations existing among the metabolic parameters of the microorganisms. This would allow structuring the Extended model as simple as possible to be user friendly yet reliable.

In this sensitivity analysis, all the yields and rates were calculated for a typical composition of municipal wastewater, assuming that the concentration of acetate fluctuates within 50-75% (mmol/mmol) and propionate within 50-25% (mmol/mmol), and the values of pH varies from 6 to 8.5 (López-Vázquez *et al.*, 2008).

The yields and rates that varied lesser than 5% when compared to the average values when influent fluctuates were considered as constant inputs to the model.

Conversion of units and use of standard notation

In the Metabolic model (Oehmen *et al.*, 2010), the units to express the concentrations are moles of carbon, N or P per litre, and the time is in terms of hours, as commonly used during laboratory experiments. On the other hand, the units in the ASM2d+TUD (Meijer, 2004) are grams of COD, N or P per cubic meter, and the time is in terms of days, as it is used in modelling of full-scale plants.

In order to make the models compatible, it was necessary to calculate the contribution of COD of each C-mmol of component and then, to convert the units of the Metabolic model (Oehmen *et al.*, 2010) from moles to grams, and from hours to days. The conversion units used are shown in the Table 24 of Appendix A.3.

Additionally, as it was done when ASM2d+TUD model was adapted, the nomenclature of parameters of the Metabolic model (Oehmen *et al.*, 2010) was updated using the standard notation proposed by Corominas et al. (2010).

Additions of some switch on/off functions, processes and components:

The Metabolic model was developed in cultures enriched with PAOs and GAOs communities focused only on the aerobic and anoxic P-removal. Nevertheless, in order to model a full-scale configuration, other variables not considered in the original model but equally relevant were included such as:

Switch on/off functions:

- Preference for certain electron acceptors: When the microorganisms are under the presence of more than one electron acceptors, they tend to prefer the one with the higher oxidation state. This preference can be modeled as a cascade reaction in which the electron acceptor with the highest oxidation state will be preferred, followed by the one with lower immediate oxidation state, and so on.
- Presence/lack of nitrogen as a nutrient and alkalinity: The formation of biomass requires a minimum concentration of ammonium nitrogen available in order to avoid that nutrient becomes a limiting factor. Other processes consume alkalinity and/or need a minimum level of alkalinity to function properly at a stable pH value.

Processes:

- Uptaking of N as a source of nutrient: The microbial growth consumes nitrogen and incorporates it in the biomass. The N-uptake depends on the growth and the fraction of N in the biomass.

Components:

- Alkalinity: The consumption or release of alkalinity was calculated according to the concept described in the literature (Metcalf&Eddy *et al.*, 2003) and included in the composition matrix.

Mass and charges balance

As it was done when ASM2d+TUD model was adapted, the consistency of the new matrix of the adapted Metabolic model was tested through executing a mass and charges balance.

3.3.3 Integration of the new processes into the existing matrix

Once the models were compatible between each other, it was possible to combine them by sharing the common components in order to avoid their duplication. The integration of the models described below is shown schematically in Figure 8.

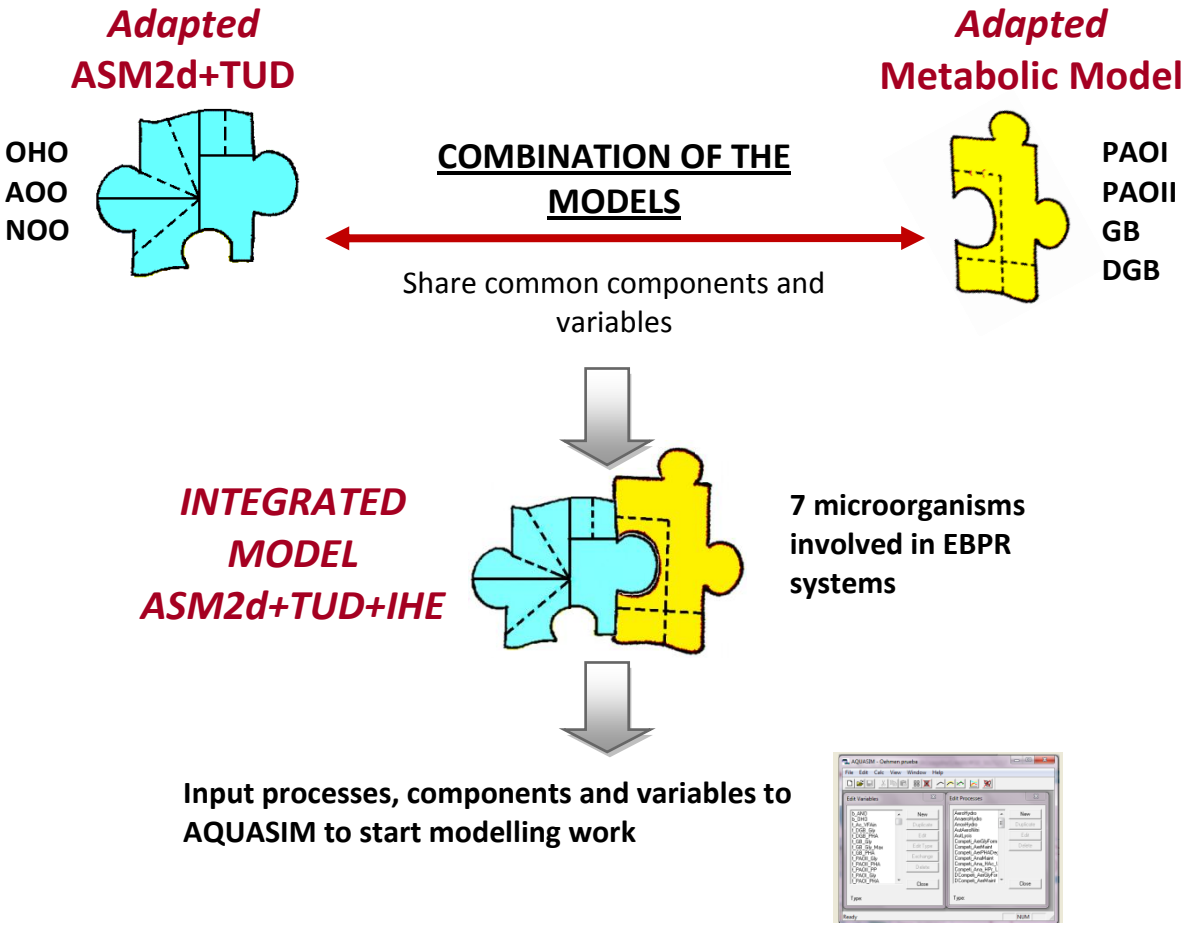


Figure 8: Joining the models

3.4 Modelling

This part of the thesis had the objective to present an example of how the different parts of the Extended model (proposed in this thesis) can be tested before the model is calibrated and validated using an integrated full-scale case study.

The simulations of the laboratory experiments were made without any change in the constant rates of the model, considering that this step is not a calibration or validation. The simulations presented in this thesis were done only to test part of the model, probing that the Extended model proposed in this thesis is able to give reasonable results.

3.4.1 Laboratory data used to test the model

The laboratory results of an enriched culture of *Competibacter* (GAO-culture, Lopez-Vazquez *et al.*, 2007) and mixture culture of *Accumulibacter* and *Competibacter* (Oehmen *et al.*, 2005) were used to test the proposed model. Both cultures were grown in sequential batch reactors (SBR) under anaerobic/aerobic conditions, using acetate as the sole carbon source. The pH was controlled within a value of 7 ± 0.1. The main difference between both cultures

was the phosphate concentration in the influent. The concentration in the influent of GAO-culture was high enough to be a source of nutrient, but was low enough to limit the proliferation of PAOs. The sludge retention time for was 10 and 8 days for GAO and PAO cultures, respectively.

The cycle of the SBRs lasted 6 hours for both cultures, the times for each phase were the following:

- GAO-culture: 2.25h of anaerobic phase, 2.25h of aerobic phase and 2h for settling and decantation.
- PAO-culture: 2h of anaerobic phase, 3h of aerobic phase and 1h for settling and decantation.

The concentrations of acetate, glycogen, PHA and phosphate (PO_4) were measured along the anaerobic/aerobic phases.

3.4.2 Test of the model

The Extended ASM2d+TUD model was introduced into AQUASIM, which is a simulator software for aquatic environments (Reichert *et al.*, 1995). The results of the simulation after three times the sludge retention time were compared with the measured values in laboratory. The percent error between the estimated and the real values was calculated through the normalised root mean squared deviation (NRMSD) by using the following formula:

$$NRMSD = \frac{\sqrt{\sum_{i=1}^{n}(x_{meas,i-} - x_{pred,i})^2}}{x_{meas,max-} - x_{pred,min}}$$

Where:

n=	data points
$x_{meas,i}$ =	measured concentration of a given variable
$x_{pred,i}$ =	predicted concentration of a given variable
$x_{meas,max}$=	maximum measure concentration in the dataset
$x_{meas,min}$=	minimum measure concentration in the dataset

4 Results

This chapter present the results obtained following the procedure described in the methodology, as well as some findings and remarks that came up during the research.

4.1. Adaptation of ASM2d+TUD Model

4.1.1 Nitrification processes

The replacement of the 1-step nitrification for a 2-step nitrification was strictly necessary in order to distinguish the metabolism of PAOI and PAOII (PAOI can use NO_3^- as electron acceptor whereas PAOII cannot). Hence nitrification done by the generic Autotrophic nitrifying organisms (ANO) was split into two subprocesses (nitritification and nitratification) done by two different microorganisms: the Autotrophic nitrifying organisms (AOO) and Nitrite oxidizing organisms (NOO) respectively.

The 1-step nitrification process of the model ASM2d+TUD was replaced by the 2-step nitrification process proposed by Sin et al. (2008a). The comparison of both models is shown in Table 3.

Table 3: Comparison of 1-step and 2-step nitrification processes

NITRIFICATION PARAMETERS	Symbol	Units	1-STEP (Meijer, 2004)	2-STEPS (Sin *et al.*, 2008a)	
			$NH_4^+ \rightarrow NO_3^-$	$NH_4^+ \rightarrow NO_2^-$	$NO_2^- \rightarrow NO_3^-$
Microorganism	-	-	Autotrophic nitrifying organisms (ANO)	Autotrophic nitrifying organisms (AOO)	Nitrite oxidizing organisms (NOO)
Maximum growth rate	μ_{Max}	1/d	1.0	1.1	1.8
Decay rate for biomass (X)	b	1/d	0.15	0.15	n.c.
Half saturation for S_{O2}	K_{O2}	$gO2/m^3$	0.5	0.1	0.3
Half saturation for S_{NHx}	K_{NHx}	gN/m^3	1.0	0.4	n.c.
Half saturation for S_{NO2}	K_{NO2}	gN/m^3	n.c.	n.c.	0.1
Half saturation for S_{Alk}	K_{Alk}	mol_{HCO3}/L	0.500	0.008	0.008
Yield of X growth per S_{NO3}	Y	gCOD/gN	0.24	0.20	0.05
Correction factor due to temperature (teta)	θ	θ	$e^{-0.105}$	1.099	1.063
Half saturation for S_{PO4}	K_{PO4}	gP/m^3	0.010	n.c.	n.c.
n.c. = not considered					

As shown in the table, the maximum growth and decay rates are similar between both models. Moreover, the differences in the correction factors (θ) may allow to simulating the

accumulation of NO_2 at temperatures higher than 20-25 ^oC (Hellinga *et al.*, 1998, Hunik, 1993), which is neglected when the 1-step nitrification is applied.

One difference between the models, is that the 2-step nitrification model (Sin *et al.*, 2008a) neglects the decay of NOO to simplify the model, considering that the growth yield of NOO is very low. For the same reason, a 2-step model also neglects any phosphorus limitation used as a nutrient.

The two-step nitrification included in the Extended model will require special attention during its validation (further research needed), considering that nitrification is commonly the rate limiting process that will define the volume of the aerobic tank (Henze *et al.*, 2008, Metcalf&Eddy *et al.*, 2003). The nitrification efficiency and the mixer liquour recycle would impact the denitrification processes done by OHOs, PAOs and GAOs afterwards.

4.1.2 Denitrification processes

There was a discrepancy between denitrification in the different models: the denitrification performed by OHO in the ASM2d+TUD model is a 1-step process ($NO_x^- \rightarrow N2$) whereas the denitrification carried out by PAOs and GAOs in the Metabolic model (Oehmen *et al.*, 2010) is assumed to be a 3-step process ($NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$). Thus, there were two alternatives to deal with this conflict. The first one was to have the minimum number of compounds required to distinguish the metabolism of PAOI and PAOII, assuming a 2-step denitrification process ($NO_3^- \rightarrow NO_2^- \rightarrow N_2$) for OHO, PAOs and GAOs, neglecting any accumulation of N_2O. The second option was to extend the ASM2d+TUD model from a 1-step process to a 3-step process, as stated in the Metabolic model (Oehmen *et al.*, 2010)**.**

The last option was selected due to the increasing need in assessing the potential accumulation of subproducts like N_2O. Certain conditions like high temperatures, inhibitory compounds, low oxygen concentration in the aerobic reactor, low retention times, etc., can cause the accumulation of intermediate compounds, which is a big concern due to its contribution to the greenhouse effect (Schulthess & Gujer, 1996).

The 1-step denitrification process of the model ASM2d+TUD was replaced by the 3-step process from the study carried out by Schulthess & Gujer (1996). Another research that modelled the denitrification as a 2-step process (Sin & Vanrolleghem, 2006) considering NO_2^- as the only intermediate that had a high similarity with ASM2d+TUD model. However, it was not used because it was not a 3-step model. The comparison between the different denitrification processes mentioned above is shown in the Table 4:

Table 4: Comparison of 1, 2 and 3-step denitrification processes

			1-STEP (Meijer, 2004)	**2-STEP** (Sin & Vanrolleghem, 2006)		**3-STEP** (Schulthess & Gujer, 1996)		
DENITRIFICATION			**$NO_3^- \rightarrow N_2$**	**$NO_3^- \rightarrow NO_2^-$**	**$NO_2^- \rightarrow N_2$**	**$NO_3^- \rightarrow NO_2^-$**	**$NO_2^- \rightarrow N_2O$**	**$N_2O \rightarrow N_2$**
Maximum growth rate of X_{OHO} under anoxic cond.	$\mu_{OHO,Max,Ax}$*	1/d	4.8	1.2	1.4	1.27	1.34	3.21
Yield for X_{OHO} growth	Y_{OHO}	g X_{OHO}/g XC_B	0.63	0.63	0.63	0.67	0.67	n.c.
Half saturation parameter for S_{O2}	$K_{O2,OHO}$	g S_{O2}/m^3	0.35	0.20	0.20	0.09	0.10	0.03
Half saturation parameter for $S_{NOx,or\ SN2O}$	$K_{NOx,OHO}$	gN/m^3	0.50	0.91	0.15	0.25	0.81	0.005
Inhibition of S_{NO2} accum in denitrification		g N/m^3	n.c.	n.c.	n.c.	12	11.3	17
Half saturation parameter for S_F	$K_{SF,OHO}$	g S_F/m^3	4	5	5	n.c.	n.c.	n.c.
Half saturation parameter for S_{VFA}	$K_{Ac,OHO}$	g S_{Ac}/m^3	4	n.c.	n.c.	5.0	1.5	2.0
Half saturation parameter for S_{NHx}	$K_{NHx,OHO}$	g S_{NHx}/m^3	0.05	0.05	0.05	n.c.	n.c.	n.c.
Half saturation parameter for S_{PO4}	$K_{PO4,OHO}$	g S_{PO4}/m^3	0.01	n.c.	n.c.	n.c.	n.c.	n.c.
Half saturation parameter for S_{Alk}	$K_{Alk,OHO}$	mol HCO_3^-/m^3	0.1	n.c.	n.c.	n.c.	n.c.	n.c.
Endogenous respiration rate of X_{OHO}	m_{OHO}	1/d	0.4	0.4	0.4	n.c.	n.c.	n.c.
Teta	θ	-	$e^{-0.069}$	1.072	1.072	n.c.	n.c.	n.c.

*Calculated assuming $n_{\mu OHO,Ax}$= 0.8

* n.c.= not considered in the model

From the table, it can be seen that there are important differences between the models. The 3-step denitrification model (Schulthess & Gujer, 1996) does not consider the impact of temperature. Additionally, the variation in the maximum growth rates, different values (or absence) of some half-saturation parameters (K), the lack/presence of inhibition function due to NO_2^-, etc. Sin *et al*. (2008b) reported that the inconsistencies between the models regarding nitrification and specially denitrification are common. Often, since one parameter is conditional to the other, the model can reproduce a similar behaviour with different combinations of parameter values. For example, in this case, the 1-step denitrification process has a higher maximum growth rate compared to the multi-step denitrification processes. Nevertheless, it also includes more K parameters that in the end, would reduce the process rate.

It is important to remark that inhibition due to the presence of NO_2^- included in the 3-step denitrification model of (Schulthess & Gujer, 1996) was not incorporated in the Extended model (this thesis), mainly because inhibition mechanisms require better understanding and there is no common agreement in literature regarding the inhibition coefficients (Sin *et al.*,

2008a)**.** The different inhibitory concentration reported in the literature are shown in Table 5.

Table 5**: Overview of the response of PAO to nitrite under different electron acceptor** (Sin *et al.*, 2008a)

	Operation strategy	**Type of reactor/wastewater**	**Aerobic P-uptake**	**Anoxic P-uptake**
Meinhold *et al.*, 1999	Anoxic/ aerobic	Pilot-scale BPR plan fed with real wastewater	10-15 mg NO_2-N/L	5-8 mg NO_2-N/L
Ahn *et al.*, 2001	Anaerobic/ aerobic	Lab-scale SBR fed with real wastewater	Not tested	No inhibition (up to 40 mgN/L)
Lee *et al.*, 2001	Anaerobic/ aerobic/ anoxic/ aerobic	Lab-scale SBR fed with synthetic wastewater, BNPR plant	Not tested	No inhibition (up to 10 mgN/L)
Hu *et al.*, 2003	Anaerobic/ anoxic	Lab-scale SBR fed with synthetic wastewater, BNPR plant	Not tested	No inhibition (up to 35 mgN/L)
Saito *et al.*, 2004	Anaerobic/ anoxic/ aerobic	Lab-scale SBR fed with real wastewater, BPR	2 mgNO_2-N/L partial; 6 mg NO_2-N/L complete inhibition	12 mg NO_2-N/L
Yoshida *et al.*, 2006	Anaerobic/ aerobic and Anaerobic/ anoxic	Lab-scale SBR fed with synthetic wastewater, BPR	1 mg NO_2-N/L for A/O; 3 mg NO_2-N/L for A2O sludge	Not tested
Soejima *et al.*, 2006	Anaerobic/ aerobic/ anoxic/ SBR	Lab-scale SBR fed with synthetic wastewater, BNPR plant	Inhibition reported but not quantified	Not tested
Sin *et al.*, 2008a	Anaerobic/ aerobic/ anoxic/ aerobic SBR and Anaerobic/ aerobic/ anoxic/ aerobic	Pilot-scale SBR and MBR fed with synthetic wastewater; BNPR plant	5 mg NO_2-N/L SBR sludge; 10 mg NO_2-N/L MBR sludge	No inhibition (up to 20 mgN/L)

In the case of the 3-step approach included in this model, the inhibition constant for NO_2^- is between 11 and 17 mgN/L, but this high accumulation of intermediaries is not expected in the hypothetical case studies used in this thesis.

Although it has been noticed that inhibition due to nitrite is pH dependant, which suggest that the free nitrous acid (HNO_2) is the real inhibitory compound (Sin *et al.*, 2008a), the inhibition coefficients require further discussion and research, which is not in the scope of this study.

Worthwhile mentioning is that despite that the 3-step denitrification model identifies the important mechanisms of nitrous oxide accumulation with denitrifying activated sludge (Schulthess & Gujer, 1996), it was calibrated with readily degradable substrate in excess and in absence of nitrifiers, so it cannot be used to predict nitrous oxide emission at a full scale wastewater treatment plant. Nonetheless, that model was used as a first approach to

estimate the production of N_2O in the system, taking into consideration that the modelling of greenhouse gases production like NO and N_2O is increasingly demanded and there is still a need to formulate reliable models regarding its mechanisms (Gürkan Sin, Kaelin, *et al.*, 2008).

4.1.3 Corrected mass and charges balance

The updated mass and charges balance is reported in the Appendix A.6 of this thesis.

4.2 Adaptation of Metabolic Model

4.2.1 Sensitivity analysis

As shown in Figure 9, the acetate and propionate taken up by PAOs and GAOs during anaerobic process are stored in different kinds of compounds, identified as poly-β-hydroxybutyrate (PHB), poly-β-hydroxyvalerate (PHV) and poly-β-hydroxy-2-methylvalerate (PH2MV). The type of compound stored depends on the microorganism, the VFA taken up (acetate or propionate) and in some cases the pH. The storage compound depends on pH when PAOs take up propionate and GAOs take up acetate.

In order to simulate the complexity of these processes, the metabolic model (Oehmen *et al.*, 2010) calculates the stoichiometry based on the different internal storage compounds (PHB, PHV and PH2MV). During the estimations, several metabolic parameters linked to the influent wastewater composition are involved like the percentage of Acetyl-CoA and Propionyl-CoA in PHA (λ and β respectively), as well as the ATP needed for biomass synthesis from Acetyl-CoA and Propionyl-CoA (K_1 and K_2 respectively). In addition, other metabolic parameters linked to oxic or anoxic conditions are involved, such as the ATP produced per NADH oxidized aerobically or anoxically (δ and δ_N respectively), as well as the aerobic and anoxic phosphate transport coefficient (ε and ε_N respectively). Furthermore, the fluctuation in pH values significantly impact some metabolic parameters like the ATP necessary to transport VFA through cell membrane (α).

Results when pH wastewater composition change

The stoichiometric and kinetic parameters were calculated for a typical range of pH in municipal wastewater, which varies between 6.0 and 8.5. Some parameters of PAOs and GAOs were affected by pH in a non-linear proportion. The parameters that depended on pH corresponded to the VFA uptake under anaerobic conditions. The difference between the values when pH is neutral and when pH is extreme fluctuated significantly, up to 90%.

The Figure 10 shows how the stoichiometry and kinetics values of anaerobic VFA uptake are affected by pH, depending on the VFA (acetate and propionate) and the microorganism (PAOs and GAOs). The latter explains why pH play a key role in the competition between PAOs and GAOs.

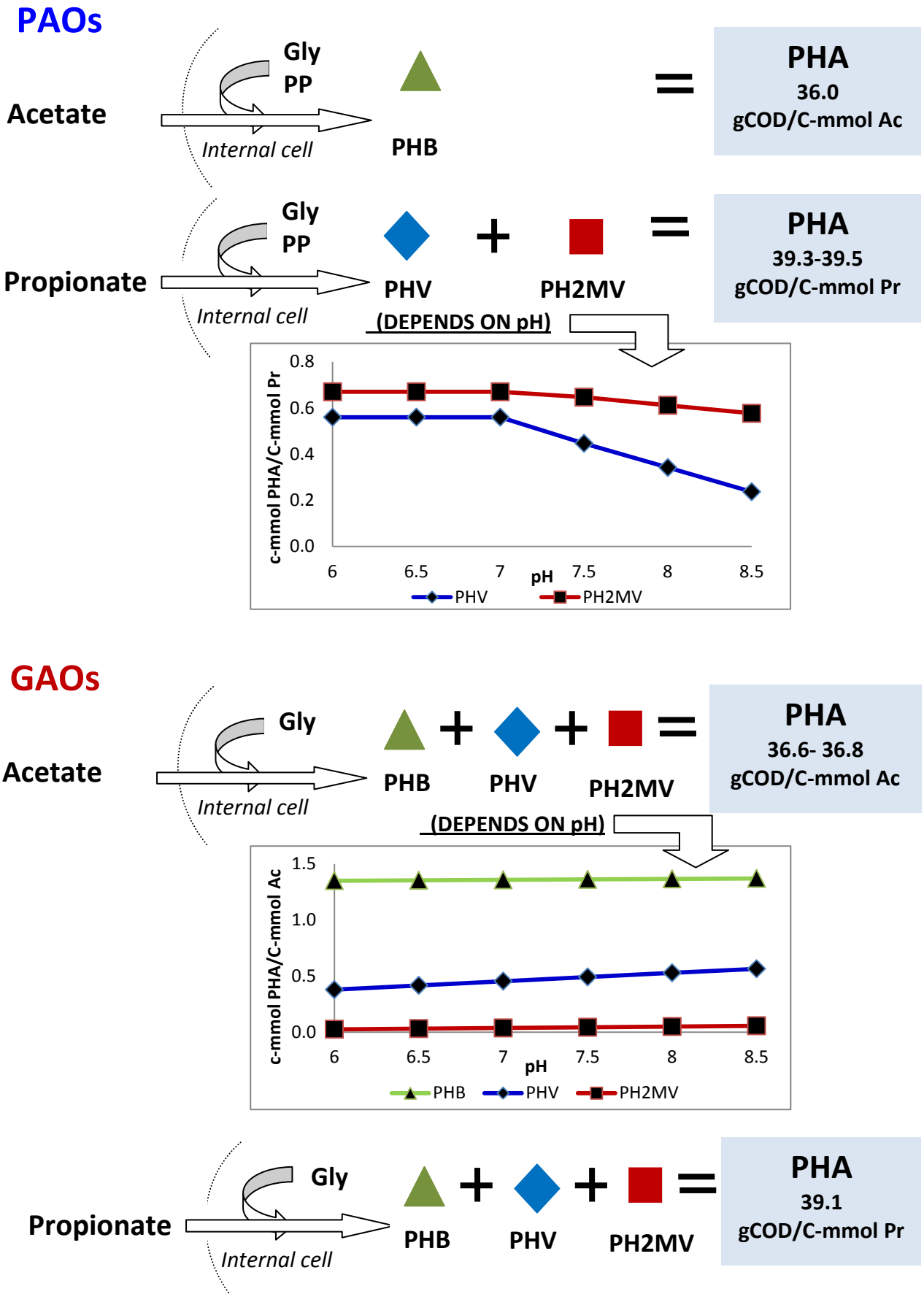
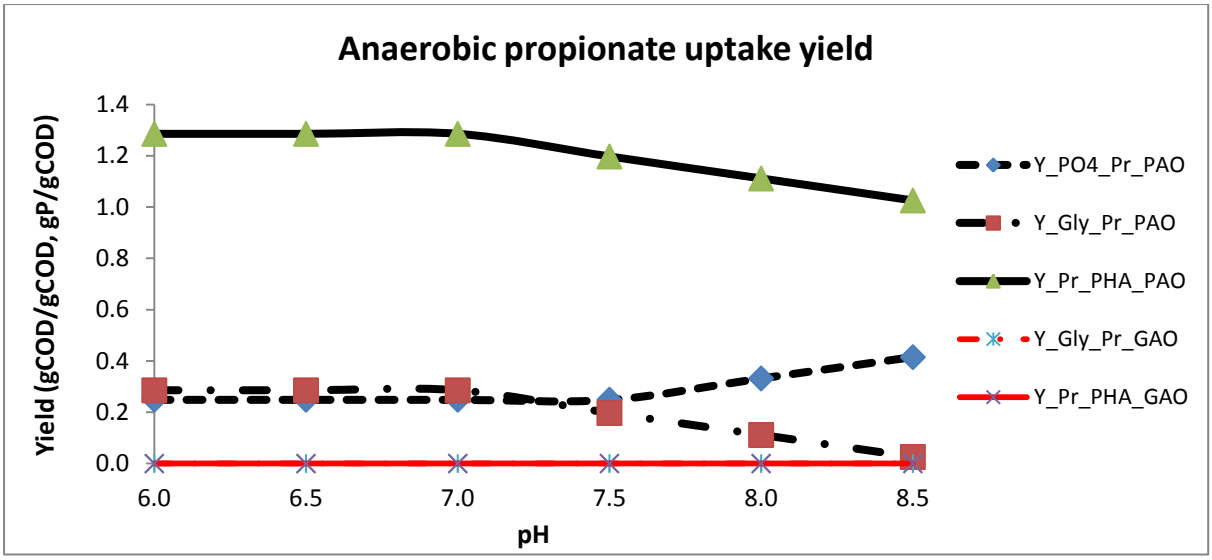
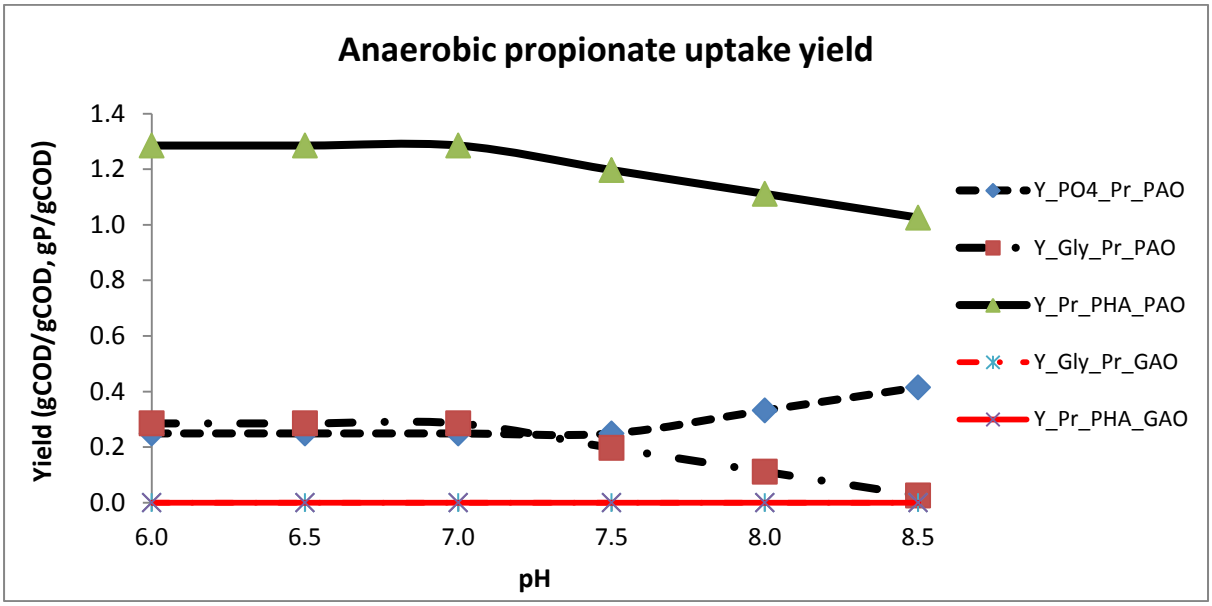


Figure 9: Internal storage compuounds depending on influent, pH and microorganism





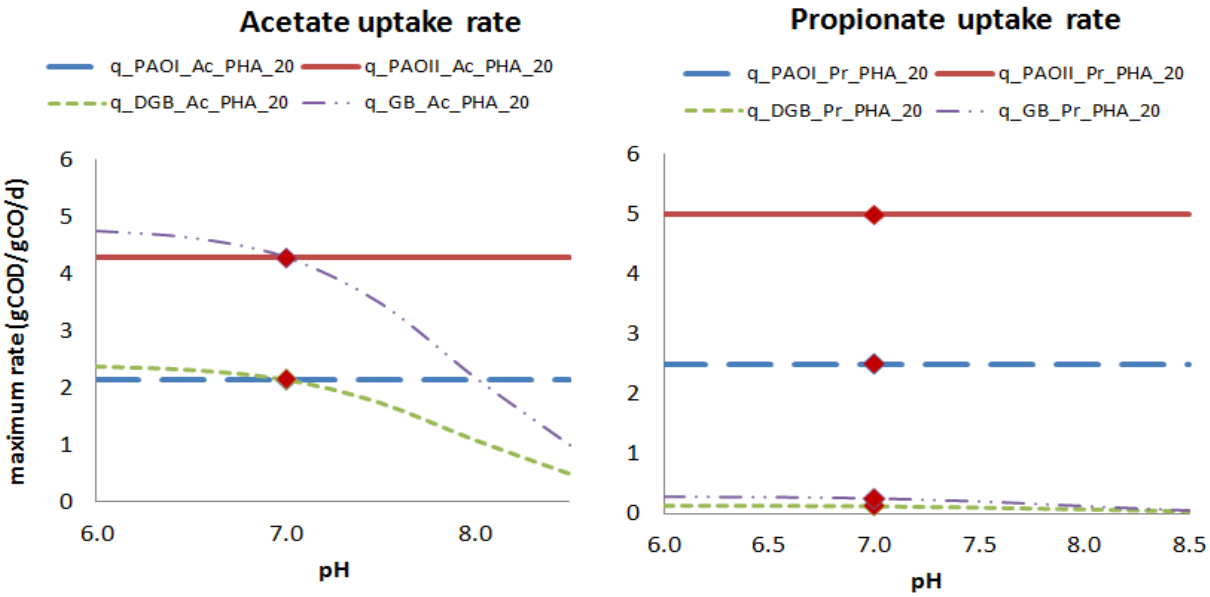


Figure 10: Impact of pH on the yields and rates of anaerobic processes of PAOs and GAOs

Results when influent wastewater composition change

The stoichiometric and kinetic parameters were calculated for a typical concentration of municipal wastewater, assuming that VFA is composed only by acetate and propionate. The minimum concentration of acetate was 50% and the maximum was 75% in terms of moles.

A large number of stoichiometric parameters (70%) and some kinetic values (13%) were affected by the influent wastewater composition. Any change in the influent wastewater composition has an impact on the balance of the internal storage compounds (see Figure 11), which affects the stoichiometry during anoxic and aerobic phases afterwards.

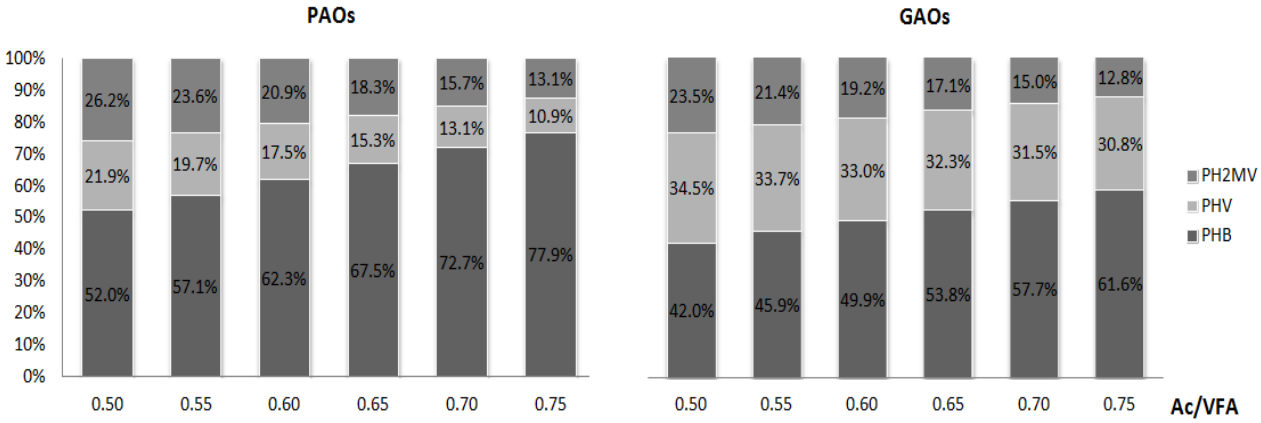


Figure 11: Composition of PHA when wastewater composition Ac/VFA fluctuates (pH 7)

Nevertheless, the outcomes of the sensitivity analysis showed that the impact of wastewater composition regarding the different expected concentrations and fractions of acetate and propionate over the affected parameters was minimal. Although the difference in parameter values when the influent in only acetate or only propionate can change up to 20%, the difference between the average and the extreme values for municipal wastewater never exceeded 3% (see Figure 12 and Table 6).

The reason is that although the balance of internal compounds changes when the influent composition fluctuates, the contribution of each C-mmol of PHA in terms of grams COD is very stable (between 36 and 39.5 gCOD/C-mmol VFA).

In order to simplify the model when the influent is municipal wastewater, any impact in the parameters values due to a change in PHA composition when pH fluctuates was neglected.

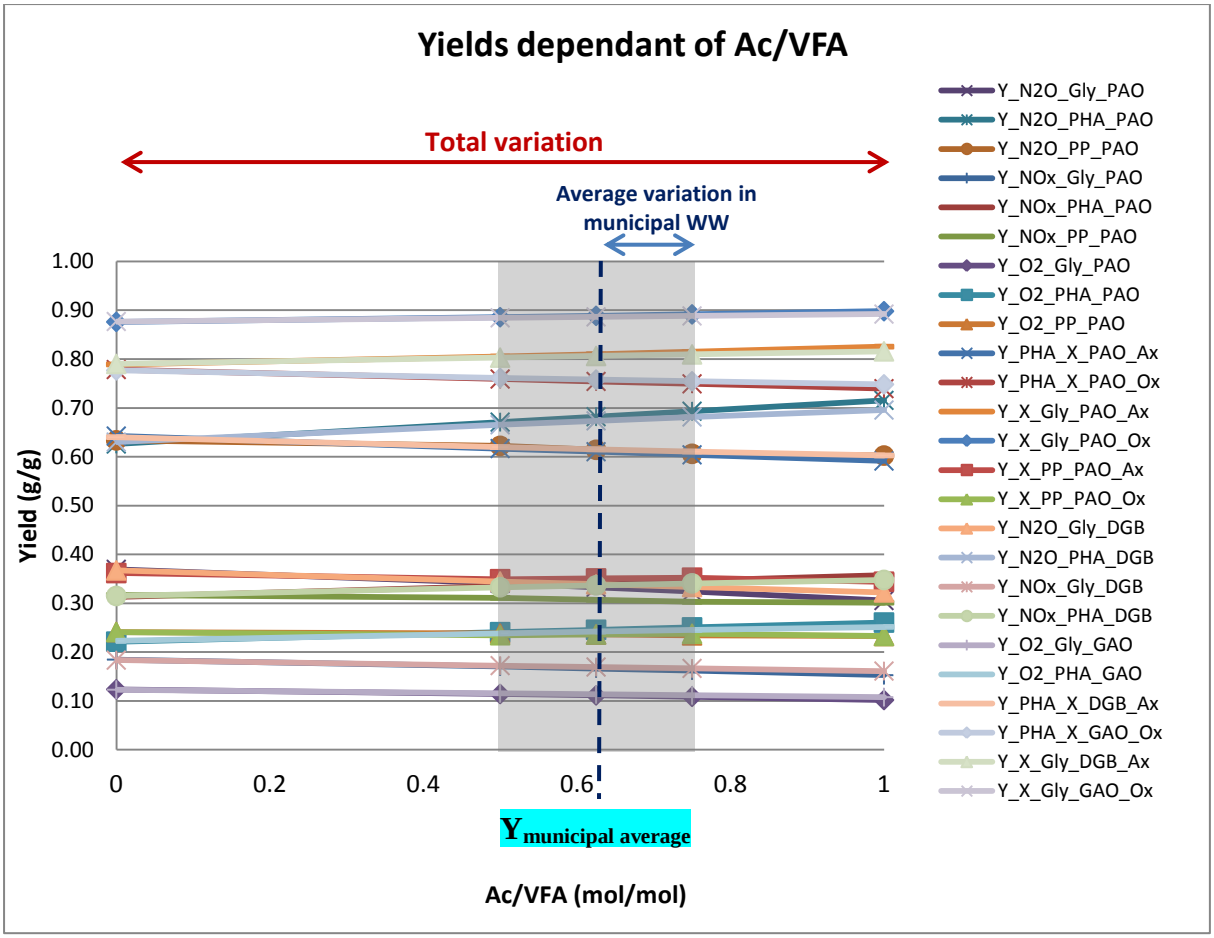


Figure 12: Yield values when wastewater composition Ac/VFA fluctuates (pH 7)

Table 6: Variation of yields depending on influent composition

Notation	Description	Total variation in municipal wastewater (%)*	Average variation in municipal wastewater (%)**
Stoichiometry *Accumulibacter* I and II (PAOs)			
$Y_{N2O\_Gly,PAO}$	Nitrous oxide not consumed per PHA degraded due to glycogen formation	**19%**	**2.6%**
$Y_{N2O\_PHA,PAO}$	Nitrous oxide consumed per PHA degraded for PAO biomass synthesis	**13%**	**1.6%**
$Y_{N2O\_PP,PAO}$	Nitrous oxide consumed per PHA degraded due to PP formation	**5%**	**1.4%**
$Y_{NOx\_Gly,PAO}$	Nitrate/ Nitrite not consumed per PHA degraded due to glycogen formation	**19%**	**2.6%**
$Y_{NOx\_PHA,PAO}$	Nitrate/Nitrite consumed per PHA degraded for PAO biomass synthesis	**13%**	**1.6%**
$Y_{NOx\_PP,PAO}$	Nitrate/Nitrite consumed per PHA degraded due to PP formation	**5%**	**1.3%**
$Y_{O2\_Gly,PAO}$	Oxygen not consumed per PHA degraded due to glycogen formation	**20%**	**2.7%**
$Y_{O2\_PHA,PAO}$	Oxygen consumed per PHA degraded for PAO biomass synthesis	**16%**	**2.0%**
$Y_{O2\_PP,PAO}$	Oxygen consumed per PHA degraded due to PP formation	**3%**	**0.8%**

Table 6: Variation of yields depending on influent composition (continue)

Notation	Description	Total variation in municipal wastewater (%)*	Average variation in municipal wastewater (%)**
$Y_{PHA\_X,PAO,Ax}$	Maximum PAO biomass production per PHA degraded under anoxic conditions	**8%**	**1.1%**
$Y_{PHA\_X,PAO,Ox}$	Maximum PAO biomass production per PHA degraded under aerobic conditions	**5%**	**0.7%**
$Y_{X\_Gly,PAO,Ax}$	PAO biomass not produced due to Gly formation under anoxic conditions	**5%**	**0.6%**
$Y_{X\_Gly,PAO,Ox}$	PAO biomass not produced due to Gly formation under aerobic conditions	**2%**	**0.3%**
$Y_{X\_PP,PAO,Ax}$	PAO biomass not produced due to PP formation under anoxic conditions	**5%**	**0.5%**
$Y_{X\_PP\_PAO\_Ox}$	PAO biomass not produced due to PP formation under aerobic conditions	**3%**	**0.7%**
Stoichiometry *Competibacter* and Denitrifying *Competibacter* (GAOs)			
$Y_{N2O\_Gly,DGB}$	Nitrous oxide not consumed per PHA degraded due to glycogen formation	**13%**	**1.8%**
$Y_{N2O\_PHA,DGB}$	Nitrous oxide consumed per PHA degraded for DGB biomass synthesis	**10%**	**1.2%**
$Y_{NOx\_Gly,DGB}$	Nitrate/ Nitrite not consumed per PHA degraded due to glycogen formation	**13%**	**1.8%**
$Y_{NOx\_PHA,DGB}$	Nitrate/Nitrite consumed per PHA degraded for DGB biomass synthesis	**10%**	**1.2%**
$Y_{O2\_Gly,GAO}$	Oxygen not consumed per PHA degraded due to glycogen formation	**13%**	**1.8%**
$Y_{O2\_PHA,GAO}$	Oxygen consumed per PHA degraded for GB / DGB biomass synthesis	**12%**	**1.5%**
$Y_{PHA\_X,DGB,Ax}$	Maximum DGB biomass production per PHA degraded under anoxic conditions	**6%**	**0.8%**
$Y_{PHA\_X,GAO,Ox}$	Maximum GB / DGB biomass production per PHA degraded under aerobic conditions	**4%**	**0.5%**
$Y_{X\_Gly,DGB,Ax}$	DGB biomass not produced due to Gly formation under anoxic conditions	**3%**	**0.4%**
$Y_{X\_Gly,GAO,Ox}$	GB biomass not produced due to Gly formation under aerobic conditions	**2%**	**0.2%**

* Formula to calculate the total variation in municipal wastewater:

$$(Y_{100\%Ac} - Y_{100\%Pr}) / Y_{municipal\ av.}$$

**Formula to calculate the variation in municipal wastewater (%):

$$(Y_{75\%Ac} - Y_{municipal\ av.})/Y_{municipal\ av.}$$

Summary of sensitivity analysis

The sensitivity analysis was carried out for the 71 yields and 30 rates included in the matrix of the Metabolic Model (A. Oehmen *et al.*, 2010). Some of the parameters were constant (independent values) and others were affected by influent composition (relation of acetate and propionate) or pH. The Table 7 shows a summary of the parameters involved in the sensitivity analysis and the Tables 8 and 9 illustrates the yields in the matrix affected by pH and influent VFA composition.

Table 7: Yields and rates dependant of influent composition and pH

Parameter	Microorganism	Constant	Affected by:		TOTAL
			Influent	pH	
Stoichiometry (yields)	PAOs (PAOI and PAOII)	3	34	6	**43**
	GAOs (GB and DGB)	6	25	0	**31**
Kinetic (process rates)	PAOs (PAOI and PAOII)	16	2	0	**18**
	GAOs (GB and DGB)	7	2	2	**11**

Based on the results of the sensitivity analysis, it was decided to neglect the effect of the influent composition in the parameters and consider the average values of the parameters as constant coefficients. On the other hand, in order to consider the impact of pH in the parameters, it was decided to introduce direct formulas that relate pH with the kinetic and stoichiometry values affected (see Table 26 of Appendix A.3).

All the calculations inside the model that involved metabolic parameters and the different storage compounds were removed, and so, PHB, PHV and PH2MV could be grouped as only poly-β-hydroxyalkanoate (PHA), assuming an average contribution of gCOD per C-mmol of VFA.

The sensitivity analysis made possible to avoid the calculations of several metabolic parameters that would make the model more complicated leading to similar results without compromising the accuracy and reliability of the model.

Table 8: Stoichiometry of PAO affected by pH and influent VFA composition

	#	Accumulibacter	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{PAOI}	X_{PAOI_PHA}	X_{PAOI_Gly}	X_{PAOI_PP}	S_{Alk}
Anaerobic processes DPAO	17	Anaerobic acetate uptake														
	18	Anaerobic propionate uptake														
	19	Anaerobic maintenance (PP)														
Aerobic processes DPAO	20	Aerobic PHA degradation														
	21	Aerobic glycogen production														
	22	Aerobic Poly-P formation														
	23	Aerobic maintenance														
NO3 reduction processes DPAO	24	Anoxic PHA degradation (on NO_3^-)														
	25	Anoxic glycogen production (on NO_3^-)														
	26	Anoxic Poly-P formation (on NO_3^-)														
	27	Anoxic maintenance (on NO_3^-)														
NO2 reduction processes DPAO	28	Anoxic PHA degradation (on NO_2^-)														
	29	Anoxic glycogen production (on NO_2^-)														
	30	Anoxic Poly-P formation (on NO_2^-)														
	31	Anoxic maintenance (on NO_2^-)														
N2O reduction processes DPAO	32	Anoxic PHA degradation (on N_2O)														
	33	Anoxic glycogen production (on N_2O)														
	34	Anoxic Poly-P formation (on N_2O)														
	35	Anoxic maintenance (on N_2O)														

Table 9: Stoichiometry of GAO affected by pH and influent VFA composition

	#	Competibacter	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{DGB}	X_{DGB_PHA}	X_{DGB_Gly}	S_{Alk}
Anaerobic processes DGB	57	Anaerobic acetate uptake													
	58	Anaerobic propionate uptake													
	59	Anaerobic maintenance													
Aerobic processes DGB	60	Aerobic PHA degradation													
	61	Aerobic glycogen production													
	62	Aerobic maintenance													
NO3 red. processes DGB	63	Anoxic PHA degradation (on NO_3^-)													
	64	Anoxic glycogen production (on NO_3^-)													
	65	Anoxic maintenance (on NO_3^-)													
NO2 red. processes DGB	66	Anoxic PHA degradation (on NO_2^-)													
	67	Anoxic glycogen production (on NO_2^-)													
	68	Anoxic maintenance (on NO_2^-)													
N2O red. processes DGB	69	Anoxic PHA degradation (on N_2O)													
	70	Anoxic glycogen production (on N_2O)													
	71	Anoxic maintenance (on N_2O)													

pH dependent variables

fAc_VFA dependent variables

4.2.2 Changes of kinetic expressions in Metabolic Model

Table 10 presents a summary of the adaptations done to the kinetic expressions of the Metabolic model. Some of the changes were inconsistencies between the actual model simulated in AQUASIM and the corresponding article (A. Oehmen *et al.*, 2010), some were omissions of the original Metabolic model, and others were considerations that were necessary to include due to the use of this model into an integrated one.

Table 10: Summary of the changes done to rate expression in the Metabolic Model

SWITCH FUNCTIONS	Processes	Microorganisms (processes)	Expression	Additional comments
Addition of inhibition due to presence of NO_3^- and NO_2^-	Anaerobic VFA uptake	**PAOI** (17,18) **DGB** (57,58)	$\left[1-\frac{S_{NO3}}{S_{NO3}+K_{NO3}}\right]\cdot\left[1-\frac{S_{NO2}}{S_{NO2}+K_{NO2}}\right]$	Diminishing in the efficiency of in EBPR-related systems has been reported due to the intrusion of not only O_2 but also NOx in the anaerobic stage (Hascoet & Florentz, 1985). Consequently, all the anaerobic processes were inhibited by the electron acceptors that the microorganisms can use during anoxic phase, which were not added consistently in the original Metabolic Model.
	Anaerobic maintenance	**PAOI** (19) **DGB** (59)		
Addition of inhibition due to presence of NO_2^-	Anaerobic VFA uptake	**PAOII** (36,37)	$\left[1-\frac{S_{NO2}}{S_{NO2}+K_{NO2}}\right]$	
	Anaerobic maintenance	**PAOII** (38)		
Addition of activation due to presence of nitrogen as a nutrient	Aerobic and anoxic PHA degradation (on NO_3^-, NO_2^- and N_2O)	**PAOI** (20, 24, 28, 32) **PAOII** (39, 43, 47) **GB** (54) **DGB** (60, 63, 65, 69)	$\frac{S_{NHx}}{S_{NHx}+K_{NHx}}$	The formation of biomass requires minimum ammonium nitrogen (NH_4) as a nutrient. Other processes consume alkalinity and/or need minimum level in order to function properly. Switch funtions for unfavourable conditions like these were included in the ASM2d+TUD model, but not by the Metabolic Model (Oehmen *et al.*, 2010), because in this model, the NH_4 was present in excess and nitrification did not occur. For simplicity, these factors were included in the integrated model only for PHA degradation. If this process is not active, any of the other aerobic and anoxic processes can continue.
Addition of activation due to presence of alkalinity	Aerobic and anoxic PHA degradation (on NO_3^-, NO_2^- and N_2O)	**PAOI** (20, 24, 28, 32) **PAOII** (39, 43, 47) **GB** (54) **DGB** (60, 63, 65, 69)	$\frac{S_{Alk}}{S_{Alk}+K_{Alk}}$	
Addition of activation due to presence of phosphorus as a nutrient	Aerobic and anoxic PHA degradation of GAOs on NO_3^-, NO_2^- and N_2O.	**GB** (54) **DGB** (60, 63, 65, 69)	$\frac{S_{PO4}}{S_{PO4}+K_{PO4}}$	Inhibition due to lack of phosphorus as a nutrient was included for GAOs but not for PAOs processes because the latter has Poly-P as a reserve of phosphorus, which is a competitive advantage.

Table 11: Summary of the changes done to rate expression in the Metabolic Model (*continuation*)

SWITCH FUNCTIONS	Processes	Microorganisms (processes)	Expression	Additional comments
Correction of activation due to presence of a minimum PHA fraction in biomass of PAOs and GAOs	Aerobic and anoxic PHA degradation on NO_3^-, NO_2^- and N_2O.	**PAOI** (20, 24, 28, 32) **PAOII** (39, 43, 47) **GB** (54) **DGB** (60, 63, 65, 69)	$\frac{f_{PAOI,PHA}}{f_{PAOI,PHA}+K_{fPHA}}$, $\frac{f_{GB,PHA}}{f_{GB,PHA}+K_{fPHA}}$ instead of $\frac{X_{PAOI,PHA}}{X_{PAOI,PHA}+K_{fPHA}}$, $\frac{X_{GB,PHA}}{X_{GB,PHA}+K_{fPHA}}$	The biomass concentration in the reactors can vary significantly from one system to another. Consequently, an expression of the existence of PHA reserves in terms of fraction is more reliable than absolute terms. This point was considered in the actual simulation of the Metabolic Model but was not consistent with the formulas published corresponding article.
Remotion of inhibition due to presence of N_2O	Anaerobic maintenance (PP)	**PAOII** (38)	$\left[1-\frac{S_{N2O}}{S_{N2O}+K_{N2O}}\right]$	Although N_2O is another electron acceptor that the microorganisms can use, it was assumed that there is not N_2O in the influent and any accumulation would escape to the atmosphere before entering again to the system.
RATE FUNCTIONS	**Processes**	**Microorganisms (Processes)**	**Expression**	**Additional comments**
Preference for NO_3^- over NO_2^-	All anoxic processes on NO_2^- (PHA degradation, glycogen production, poly-P formation and maintenance)	**PAOI** (28-31) **DGB** (66-68)	$\frac{S_{NO2}}{(S_{NO2}+S_{NO3})+K_N}$	Under an anoxic environment in which the microorganisms are under the presence of more than one electron acceptor, they tend to prefer the one with higher oxidation number (NO_3^-, NO_2^-, and N_2O, in that order). The preference creates a cascade-reaction effect.
Preference for NO_2^- over N_2O	All anoxic processes on N_2O (PHA degradation, glycogen production, poly-P formation and maintenance)	**PAOI** (32-35) **PAOII** (47-50) **DGB** (69-71)	$\frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_N}$	

4.2.3 Addition of components into the matrix of Metabolic Model

The incorporation of nitrogen into the biomass and the consumption or release of alkalinity are two processes and originally were not incorporated in the Metabolic model, but due to the varying conditions they should be considered at full-scale plants, following the same approach used with the ASM2d+TUD model. As a result, the new matrix of the integrated model incorporated two more columns, one for the ammonium nitrogen (S_{NHx}) and another for alkalinity (S_{Alk}).

The ammonium nitrogen was estimated considering the fraction of N in the biomass and the microbial growth:

$$S_{NHx} = i_{N\_XBio} * Y$$

$$S_{NHx} = i_{N\_XBio} * Y[=]\frac{gN}{gCOD}$$

The alkalinity was calculated by summing up the contribution of ion charges of the different species:

$$S_{Alk} = \frac{-1}{64}S_{Ac} + \frac{-1}{112}S_{Pr} + \frac{-1}{14}S_{NO3} + \frac{-1}{14}S_{NO2} + \frac{1}{14}S_{NHx} + \frac{-1.5}{31}S_{PO4} + \frac{-1}{31}X_{PAO\_PP}$$

$$S_{Alk}[=]\frac{mol_{HCO3}}{gCOD}$$

4.2.4 Changing all yields into a positive signal

The matrix of the metabolic model is easier to understand when the calculation of the parameters always result positive and then, in the matrix is added a positive signal in case of production and negative signal in case of consumption:

Production	+Y
Consumption	-Y

For this reason, all the calculations of yields were reviewed; the calculation for the ones that resulted negative were inverted and then, the signal was added in the matrix, so that all the parameters were consistently expressed as described before. The calculations of the Metabolic model that needed to change were the ones corresponding to the electron acceptor consumed per PHA degraded due to PP formation:

Table 11: Summary of the changes done to rate expression in the Metabolic Model

Parameter	Original formula	Updated formula
$Y_{O2\_PP,PAO}$	$\frac{Y_{PHA\_X}}{Y_{O2\_X} * Y_{PHA\_PP}} - \frac{1}{Y_{O2\_PP}}$	$\frac{1}{Y_{O2\_PP}} - \frac{Y_{PHA\_X}}{Y_{O2\_X} * Y_{PHA\_PP}}$
$Y_{NOx\_PP,PAO}$	$\frac{Y_{PHA\_X}}{Y_{NOx\_X} * Y_{PHA\_PP}} - \frac{1}{Y_{NOx\_PP}}$	$\frac{1}{Y_{NOx\_PP}} - \frac{Y_{PHA\_X}}{Y_{NOx\_X} * Y_{PHA\_PP}}$
$Y_{N2O\_PP,PAO}$	$\frac{Y_{PHA\_X}}{Y_{N2O\_X} * Y_{PHA\_PP}} - \frac{1}{Y_{N2O\_PP}}$	$\frac{1}{Y_{N2O\_PP}} - \frac{Y_{PHA\_X}}{Y_{N2O\_X} * Y_{PHA\_PP}}$

4.2.5 First mass and charges balance

After the sensibility analysis and unit conversion, the consistency of the new stoichiometry was tested through a mass balance of COD, N and P, as well as a charge balance. Some systematic errors were found in the Metabolic Model and corrected in the Integrated Model of this thesis. These inconsistencies were not detected preoviously because the Metabolic model use a C-mmol balance to test the stoichiometry, but not a COD balance as done in this study.

4.2.6 Correction of systematic errors in the Metabolic Model

Correction of anoxic and aerobic maintenance coefficients

The processes corresponding to aerobic and anoxic maintenance (on NO_x^- and N_2O) for all the communities did not close in the COD balance. The results for these parameters ($m_{PAO,Ox}$, $m_{PAO,NOx}$, $m_{PAO,Ox}$, $m_{GAO,N2O}$, $m_{DGB,NOx}$, $m_{DGB,N2O}$) in the Metabolic Model, compared to the ones needed to close the COD balances are up to 15 times lower. Tracking down the source of this inconsistency, a systematic error in the original formula of the Metabolic model (A. Oehmen *et al.*, 2010) to calculate these yields was found.

Nevertheless, the Metabolic Model did not use any pivot in the matrix, which made the calculation longer and difficult to compare with the ASM2d+TUD model. For this reason, the formula was simplified and all the terms in the matrix were divided into the yields for biomass production per PHA degradated (Y_{PHA_X}), making X_{PAO} the pivot component in the process.

The original formulas of the Metabolic Model and the formulas proposed in this thesis for the Metabolic Model are presented in order to close the balance, both in terms of C-mmol. Additionally, the simplified formula that was used in the integrated model is also presented, with X_{PAO} as the pivot component and the units in terms of grams of COD.

- **Original formula in Metabolic Model (C-mmol/C-mmol or N-mmol/C-mmol)**

$$m_{Ox} = Y_{PHA_X}\left(\frac{RedoxPHA}{4} * \frac{1}{Y_{PHA_X}} - \frac{RedoxBM}{4}\right) - 1$$

$$m_{NOx} = Y_{PHA\_X}\frac{4}{2}\left(\frac{RedoxPHA}{4} * \frac{1}{Y_{PHA\_X}} - \frac{RedoxBM}{4}\right) - 1$$

$$m_{N2O} = Y_{PHA\_X}\frac{4}{1}\left(\frac{RedoxPHA}{4} * \frac{1}{Y_{PHA\_X}} - \frac{RedoxBM}{4}\right) - 1$$

- **Proposed formula for the Metabolic model (C-mmol/C-mmol or N-mmol/C-mmol)**

$$m_{Ox} = Y_{PHA\_X}\left(\frac{RedoxPHA}{4}\right)$$

$$m_{NOx} = Y_{PHA\_X}\frac{4}{2}\left(\frac{RedoxPHA}{4}\right)$$

$$m_{N2O} = Y_{PHA_X}\frac{4}{1}\left(\frac{RedoxPHA}{4}\right)$$

- **Formula used in the integrated model of this thesis (gO2/gCOD or gN/gCOD)**

$$m_{Ox} = \left(\frac{RedoxPHA}{4}\right)\left(\frac{32gO2}{mol\ O2}\right)\left(\frac{C-mol\ PHA}{37gCOD}\right) = 1\frac{gO2}{gCOD}$$

$$m_{NOx} = \frac{4}{2}\left(\frac{RedoxPHA}{4}\right)\left(\frac{14gN}{mol\ N}\right)\left(\frac{C-mol\ PHA}{37gCOD}\right) = \frac{1}{i_{COD_{NO2}} - i_{COD\_NO3}} = 0.875\frac{gN}{gCOD}$$

$$m_{N2O} = \frac{4}{1}\left(\frac{RedoxPHA}{4}\right)\left(\frac{14gN}{mol\ N}\right)\left(\frac{C-mol\ PHA}{37gCOD}\right) = \frac{1}{i_{COD_{N2O}} - i_{COD\_NO3}} = 1.75\frac{gN}{gCOD}$$

<u>Correction of PHA stored per Pr taken up by PAO under anaerobic conditions</u>

Despite that the C-mmol balance did close for all pH values, the COD balance did not close for the anaerobic process of propionate uptake by PAO when the pH was higher than 7. The PHA stored per Pr taken up ($Y_{Pr\_PHA\_PAO}$), compared to the sum of propionate uptaken and the Gly consumed, had a deviation up to 17% when the pH was 8.5. As a consequence, in this thesis it was proposed a new formula to calculate $Y_{Pr\_PHA\_PAO}$ as a function of pH:

- **Original formula in the Metabolic Model (C-mmol/C-mmol)**

If pH ≤ 7.0, 1.23 $X_{PAO,PHA}$
If pH > 7.0, (3.198 - 1.28pH) $X_{PAO,PHA}$

- **Proposed formula for Metabolic model (C-mmol/C-mmol)**

If pH ≤ 7.0, 1.23 $X_{PAO,PHA}$
If pH > 7.0, (2.360 - 0.163pH) $X_{PAO,PHA}$

- **Formula used in the integrated model of this thesis (gCOD/gCOD)**

If pH ≤ 7.0, 1.285 $X_{PAO,PHA}$
If pH > 7.0, (2.495 - 0.173pH) $X_{PAO,PHA}$

4.2.7 Corrected mass and charges balance

The updated mass and charges balance is reported in the Appendix A.6 of this thesis.

4.3 Joining the models

The result of the ASM+TUD model (Meijer, 2004) and the Metabolic model (Oehmen *et al.*, 2010) was the Extended ASM+TUD model proposed in this thesis. The corresponding state variables, stoichiometry, kinetics and balances are presented in Appendix A.1 to A.8 No. __ of this thesis.

4.4 Modelling in Aquasim

Due to the considerable number of processes and microorganisms involved in the Extended model (this thesis), it is reasonable to start by testing the different parts of the model step by step, and then to do an integral validation using a full-scale WWTP as a case study.

For instance, a culture can be simulated firstly using acetate, then using propionate, and finally under a mixture of acetate and propionate. Similarly, it is easier to simulate the microbial communities one by one, and then to continue with different mixtures of cultures until all of them are included.

Although a fully validation of the model falls out of the reach of the present study, the competition between PAO and GAO using acetate as substrate was evaluated. Clearly, other parts of the Extended model (this thesis) included in the original ASM2d+TUD model (Meijer, 2004) still need to be tested (further research), such as the metabolism of PAOs and GAOs under the presence of propionate and different pH values, the two-step nitrification process, and the multi-step denitrification processes carried out by OHO, PAOs and GAOs.

4.4.1 Adjustment of yields when there is a sole carbon source

As explained in chapter 4.2.1, the yields proposed in this model were calculated after a sensitivity analysis, assuming a typical VFA composition of municipal wastewater, in which the acetate content is between 50 and 75% of the total VFA. However, the laboratory experiments used to test this model were carried out using only acetate. The inconvenience of using this data is that the difference between several yields proposed in this model and the ones resulting when acetate is the only carbon source were up to 15%. Consequently, the accumulation of the different errors along the calculations of the cycles caused that the yields proposed in the sensitivity analysis were no longer valid to simulate this kind of laboratory experiments on a sole carbon source

Taking the previous observations into account, and adding the need to to test the model for propionate as the only carbon source, it was proposed to include some formulas for the yields that presented deviations higher than 5% when the source of carbon are acetate or propionate only (see Table 12). These formulas have correlations (R^2) that varies between

0.92 and 1.00. The use of them would be only necessary during the validation of the model with laboratory data. Once the model is used on a full-scale WWTP, the formulas can be replaced for the values proposed in Table 25 of the Appendix A.4 in order to make the model simpler and faster.

Table 12: Formulas proposed for yields and rates sensitive to the use of a sole carbon source

Notation	100% Ac	100% Pr	% difference	Formula proposed f_{Ac_VFA} = fraction of Ac in VFA, (gCOD/gCOD)	Correlation R^2*
Stoichiometry *Accumulibacter* I and II (PAOs)					
$Y_{N2O\_Gly,PAO}$	0.31	0.37	19%	$0.369951+(-0.063774*f_{Ac\_VFA})$	1.000
$Y_{N2O\_PHA,PAO}$	0.72	0.63	13%	$0.62782+(0.089838* f_{Ac\_VFA})$	0.997
$Y_{N2O\_PP,PAO}$	0.60	0.63	5%	$0.634199+(-0.033258* f_{Ac\_VFA})$	0.949
$Y_{NOx\_Gly,PAO}$	0.15	0.18	19%	$0.184946+(-0.031832* f_{Ac\_VFA})$	0.999
$Y_{NOx\_PHA,PAO}$	0.36	0.31	13%	$0.313918+(0.044923* f_{Ac\_VFA})$	0.997
$Y_{NOx\_PP,PAO}$	0.30	0.32	5%	$0.317104+(-0.016674* f_{Ac\_VFA})$	0.949
$Y_{O2\_Gly,PAO}$	0.10	0.12	20%	$0.123971+(-0.021727* f_{Ac\_VFA})$	0.999
$Y_{O2\_PHA,PAO}$	0.26	0.22	16%	$0.222021+(0.039267* f_{Ac\_VFA})$	0.998
$Y_{PHA\_X,PAO,Ax}$	0.59	0.64	8%	$0.641457+(-0.051355* f_{Ac\_VFA})$	0.997
$Y_{PHA\_X,PAO,Ox}$	0.74	0.78	5%	$0.777974+(-0.039269* f_{Ac\_VFA})$	0.998
$Y_{X\_Gly,PAO,Ax}$	0.83	0.79	5%	$0.788726+(0.036421* f_{Ac\_VFA})$	1.000
$Y_{X\_PP,PAO,Ax}$	0.34	0.36	5%	$(0.641457+(-0.051355* f_{Ac\_VFA}))$ $/(1.779093+(-0.065846* f_{Ac\_VFA}))$	0.999
Stoichiometry *Competibacter* and Denitrifying *Competibacter* (GAOs)					
$Y_{N2O\_Gly,DGB}$	0.32	0.37	13%	$0.366417+(-0.045285* f_{Ac\_VFA})$	0.997
$Y_{N2O\_PHA,DGB}$	0.70	0.63	10%	$0.632557+(0.066282* f_{Ac\_VFA})$	0.989
$Y_{NOx\_Gly,DGB}$	0.16	0.18	13%	$0.183157+(-0.022551* f_{Ac\_VFA})$	0.997
$Y_{NOx\_PHA,DGB}$	0.35	0.32	10%	$0.316253+(0.033151* f_{Ac\_VFA})$	0.988
$Y_{O2\_Gly,GAO}$	0.11	0.12	13%	$0.122745+(-0.015294* f_{Ac\_VFA})$	0.998
$Y_{O2\_PHA,GAO}$	0.25	0.22	12%	$0.224143+(0.028799* f_{Ac\_VFA})$	0.989
$Y_{PHA\_X,DGB,Ax}$	0.60	0.64	6%	$0.638771+(-0.037897* f_{Ac\_VFA})$	0.988
Kinetics *Accumulibacter* I and II (PAOs)					
$m_{PAO\_PHA,Ax,20}$	0.10	0.08	12%	$0.084432+(0.010828* f_{Ac\_VFA})$	0.999
$m_{PAO\_PHA,Ox,20}$	0.10	0.09	10%	$0.08824+(0.009739* f_{Ac\_VFA})$	0.998
$q_{PAO,PHA\_Ac,Ax,20}$	7.22	7.88	9%	$7.841571+(-0.622967* f_{Ac\_VFA})$	0.933
$q_{PAO,PHA\_Ac,Ox,20}$	19.26	21.01	9%	$20.910865+(-1.66126* f_{Ac\_VFA})$	0.933
$q_{PAO,PHA\_Pr,Ax,20}$	1.20	1.31	9%	$1.306899+(-0.103811* f_{Ac\_VFA})$	0.933
$q_{PAO,PHA\_Pr,Ox,20}$	7.95	8.67	9%	$8.6257+(-0.685205* f_{Ac\_VFA})$	0.933
Kinetics *Competibacter* and Denitrifying *Competibacter* (GAOs)					
$m_{DGB\_PHA,Ax,20}$	0.09	0.08	22%	$0.085057+(0.007872* f_{Ac\_VFA})$	0.991
$m_{GAO\_PHA,Ox,20}$	0.10	0.09	9%	$0.088749+(0.00717* f_{Ac\_VFA})$	0.990
$q_{DGB\_PHA,Ax,20}$	10.30	10.99	7%	$10.939647+(-0.657371* f_{Ac\_VFA})$	0.962
$q_{GAO,PHA,Ox,20}$	26.98	28.78	7%	$28.651592+(-1.721764* f_{Ac\_VFA})$	0.925

* This correlation considers the calculation of the yields in five different fAc_VFA, all of them resulted in linear equations except $Y_{X\_PP,PAO,Ax}$

4.4.2 Adjustment of half-saturation constants.

At the beginning, it was intended to use the same half saturation constants (K) reported in the Metabolic model (Oehmen *et al.*, 2010), which were merely very low values used to turn on or off the processes. Nevertheless, the inconvenience of this change was that during the simulations, some concentrations reached zero values or switched to negative concentrations. This problem was related to the adjustment of the number of internal steps used for the simulations in AQUASIM, which was changed due to the conversion of time units from hours to days in the Extended model (proposed in this thesis).

In order to avoid the indetermination of equations that tended to stop the simulations, as well as obtaining negative results, it was necessary to change some K constants, which were higher values compared to the ones used in the Metabolic model (Oehmen *et al.*, 2010), but did not exceeded the K values used in the ASM2d+TUD model (Meijer, 2004).

In other cases, it was considered more convenient to use directly the K values proposed in the ASM2d+TUd model (Meijer, 2004) because they were based on more studies.

The following table present the K values used in the simulations of this thesis compared to values used in the Metabolic model (Oehmen *et al.*, 2010) and the ASM2d+TUD model (Meijer, 2004).

Table 13: Comparison of K values used in the simulation

Notation	Coefficient for	Units	Metabolic m. (Oehmen *et al.*, 2010)	ASM2d+TUD (Meijer, 2004)	Extended m. (this thesis)
K_{S_Ac}	Acetate	$gCOD/m^3$	0.03	4	**4**
K_{S_Pr}	Propionate	$gCOD/m^3$	0.04	n.c.	**4**
K_{O2}	Oxygen	$gO2/m^3$	0.32	0.2	**0.2**
K_{fPHA}	PHA fraction	gCOD /gCOD	0.01	0.2	**0.05**
$K_{PO4,GAO}$	Phosphate as nutrient	gP/m^3	0.32	0.02	**0.3**
$K_{PO4,PAO}$	Phosphate for PAOs	gP/m^3	3.2	0.01	**3.2**
K_{VFA}	Volatile Fatty Acids	$gCOD/m^3$	n.c.	n.c.	**1**
K_{PP}	Poly-P concentration	gP/m^3	0.31	0.01	**0.3**
K_{Gly}	Glycogen concentration	$gCOD/m^3$	0.32	0.01	**0.3**
K_{PHA}	PHA concentration	$gCOD/ m^3$	37	0.01	**10**
$K_{f\_PHA\_max}$	Difference between $f_{PHA,Max}$ and f_{PHA}	gCOD/gCOD	0.05	n.c.	**0.05**
$K_{f\_PP\_max}$	Difference between $f_{PP,Max}$ and f_{PP}	gP/gCOD	0.009	0.01	**0.01**
$K_{f\_Gly\_max}$	Difference between $f_{Gly,Max}$ and f_{Gly}	gCOD/gCOD	0.009	0.01	**0.01**
n.c. = not considered in the model					

4.4.3 Results of the simulation of GAO culture

The results of the simulation of enriched GAO-cultures are shown in Figure 13.

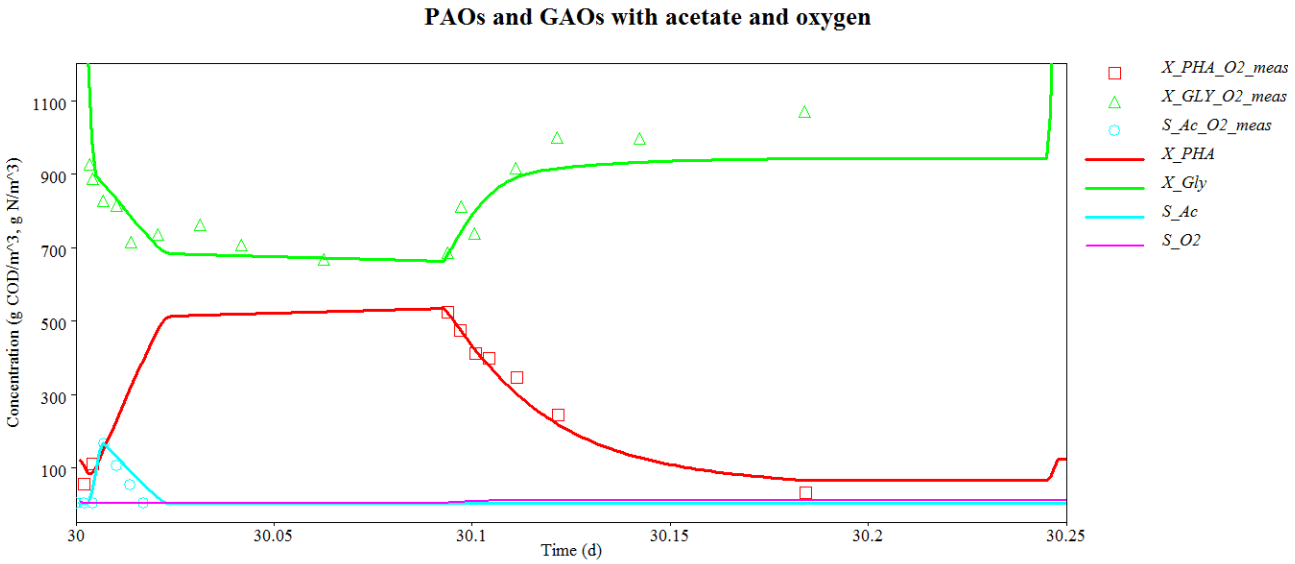


Figure 13: Simulation of enriched GAO culture and experimental data (Lopez-Vazquez *et al.*, 2007)

The percentage deviation of the simulation compared with the experimental data were 13% for acetate, 24% for glycogen and 6% for PHA.

Considering that FISH techniques during the experiments confirmed that GAO were the dominant microorganism community in the SBR (Lopez-Vazquez *et al.*, 2007), it was assumed during the simulations that all the biomass present was composed by GAOs.

4.4.4 Results of the simulation of PAO culture

The results of the simulation of enriched PAO-cultures are shown in Figure 14.

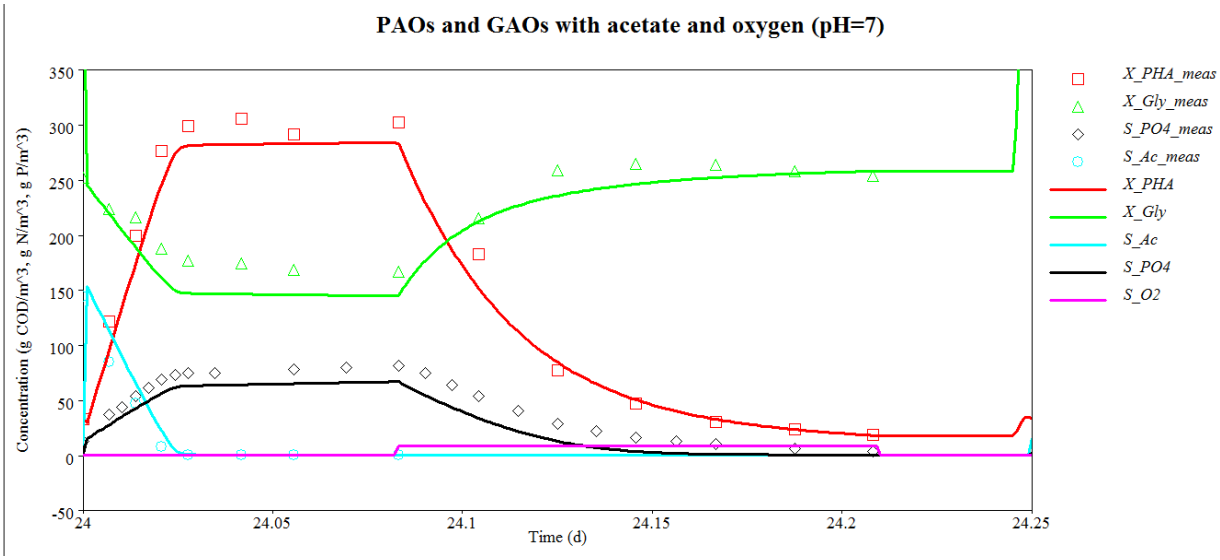


Figure 14: Simulation of enriched PAO culture and experimental data (Oehmen *et al.*, 2005).

The percentage errors of the simulation compared with the experimental data were 11% for acetate, 19% for glycogen and 7% for PHA and 17% for phosphate.

For the simulations, it was assumed that all the biomass present was composed by a mixture of PAOs and GAOs. Although FISH tests of the culture reported 11% of occurrence of other communities besides PAOs and GAOs, the predicted balance between *Accumulibacter* and *Competibacter* were similar to the ones measured (Oehmen *et al.*, 2005).

Table 14: Predicted and measured occurrence of PAOs and GAOs in enriched PAO-culture

Community	Predicted in the Extended model (this thesis)	Measured (Oehmen *et al.*, 2005) -Adjusted-	Measured (Oehmen *et al.*, 2005)
	$\frac{PAOs\ or\ GAOs}{PAOs + GAOs}$		$\frac{PAOs\ or\ GAOs}{total\ biomass}$
Accumulibacter (PAOs)	77%	73%	65 %
Competibacter (GAOs)	23%	27%	24%
Other microbial communities	-	-	11%

4.4.5 Reliability of simulation using the Extended model

The simulations of the laboratory experiments had the main purpose of testing parts of the Extended model, probing that it can provide reasonable profiles of EBPR systems. These simulations were not part of a calibration or validation step, so none of the constant rates or any other variables was changed (except for the K values for the reasons mentioned above).

The rates proposed originally in the Metabolic model (Oehmen *et al.*, 2010) remained exactly the same, despite the fact that the final calibration and validation of the referenced model was based on anaerobic/anoxic cultures (Carvalho *et al.*, 2007, Zeng *et al.*, 2003a, Zeng *et al.*, 2003b) that were different from the ones tested in the simulations of this thesis (Lopez-Vazquez *et al.*, 2007, Oehmen *et al.*, 2005) .

Considering the simulations obtained for GAO and PAO cultures, it can be concluded that at least for this part of the model (the competition between PAOs and GAOs for acetate under anaerobic/aerobic conditions) the Extended model is reliable.

For further calibration steps, it is suggested to evaluate the following kinetic parameters in order to obtain a more accurate profile: the VFA uptake rates (q_{VFA_PHA}), the glycogen production rates (q_{GLY}), the PHA degradation rates (q_{PHA}) and poly-P formation rates ($q_{PO4\text{-}PP}$).

5 Discussion

5.1 Comparison of PAO processes

Because all the PAO processes of ASM2d+TUD model were replaced by the processes of PAOI and PAOII of the Metabolic model, it was considered appropriate to analyse and compare the approach used in both models in order to corroborate the coherence of the parameters and also to foresee the impact on the modelling results due to this replacement.

5.1.1 Model

The model structure regarding the P removal processes are shown in Figures 15 and 16 for ASM2d+TUD and the adapted Metabolic Model in this thesis, respectively. The similarities and differences are listed below:

Similar kinetic structure

The kinetic structure is similar in both models, which follows the approach proposed by (Murnleitner *et al.*, 1997), similar to the endogenous respiration and maintenance concept (Henze *et al.*, 1999, Lopez *et al.*, 2006). This mathematical method assumes firstly that all the PHA consumed during aerobic/anoxic stages is converted into biomass and then, the energy required for PP, glycogen and maintenance is calculated and subtracted from the biomass. This is an indirect mathematical method to calculate PAO growth; showing that for competition purposes, PAOs and GAOs rely in their storage ability more than growth (Brdjanovic, van Loosdrecht, *et al.*, 1998).

Different PAO microbial populations involved

The main difference between ASM2d+TUD and the Metabolic Model is the number of microbial population involved. ASM2d+TUD model assumed only one PAO population with both aerobic and anoxic metabolism whereas the Metabolic Model assumes two different PAO populations (PAOI and PAOII) with different capacities in their denitrification metabolism.

Different number of steps for denitrification processes

Due to the differences between the metabolic capacities of the communities incorporated, the Metabolic model included a 3-step denitrification processes while the ASM2d+TUD model has a 1-step denitrification process.

Different approach to simulate the anoxic storage of VFA

The models have two different approaches to simulate the anoxic storage of VFA observed by Kuba *et al.* (1994), who reported that PAOs are able to release PO_4^{+3}, take up acetate and synthesize PHB simultaneously under the presence of NO_3^- and acetate. The ASM2d+TUS model includes a specific process to model this behaviour, whereas the Metabolic model can simulate this through the division of two PAO communities, in which one can use NO_3^- as an electron acceptor (PAOI) and the other cannot (PAOII). This causes that when there is only NO_3^- present as an electron acceptor, PAOI activates its anoxic metabolism and consumes NO_3^-, whereas PAOII activates its anaerobic metabolism and consumes VFA.

Different influent for PAOs

The ASM2d+TUD model assumed that all the influent VFA was acetate, whereas the Metabolic model considered that the influent is a mixture of acetate and propionate that would affect the stoichiometric and kinetic values.

Incorporation of competitors and pH impact

The Metabolic Model included four microbial communities that compete with PAOs (the integrated model proposed in this thesis includes only two of them). This model also considered the effect of pH on the stoichiometry and kinetic values. These factors (occurrence of competitors and pH values) were not included in the ASM2d+TUD model. Nevertheless, under certain conditions these factors can highly impact the efficiency of P removal.

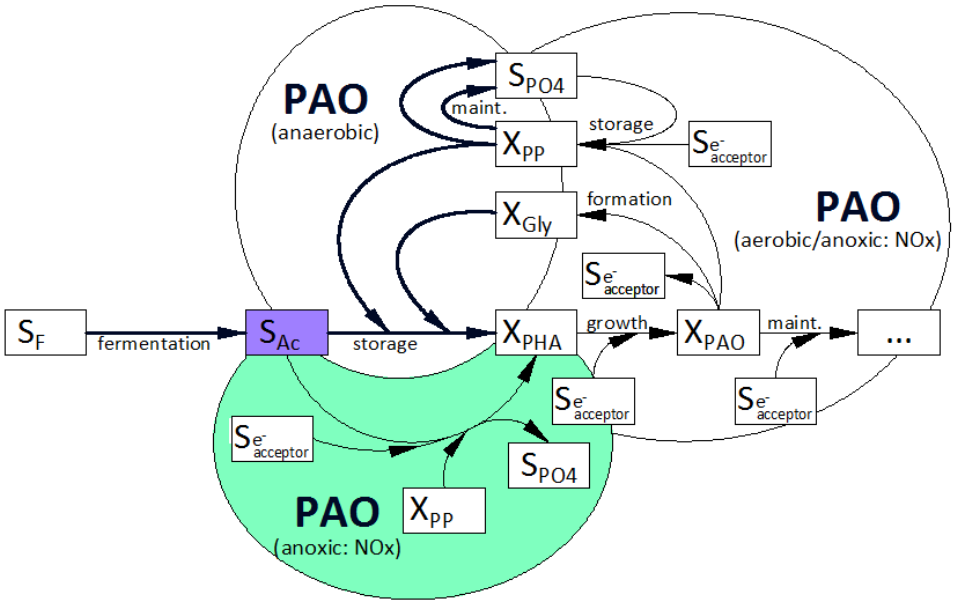


Figure 15: Interactions in the components for PAO microorganism in ASM2d+TUD model

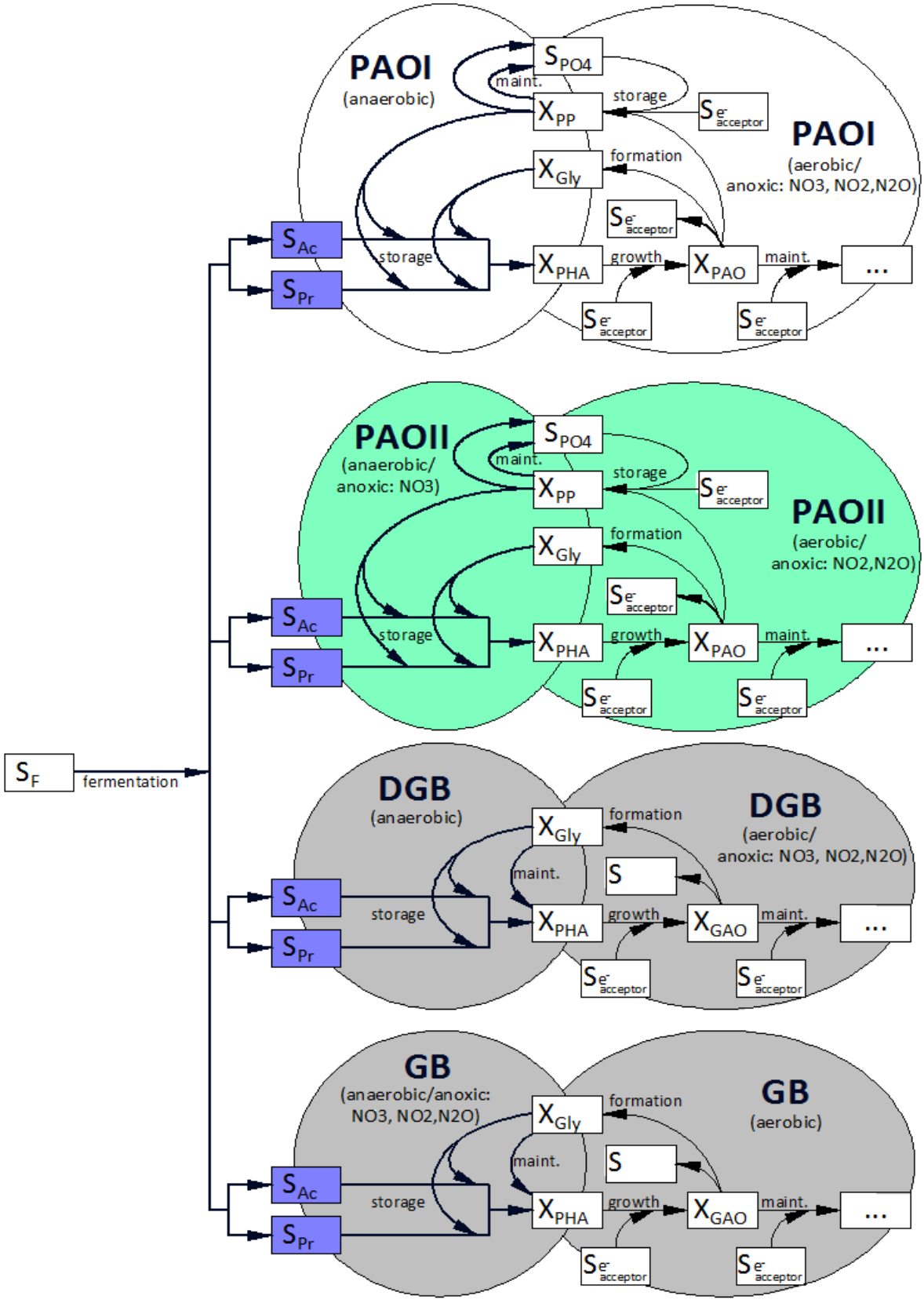


Figure 16: Interactions in the components for PAO microorganism in the adapted Metabolic Model

5.1.2 Processes

The Extended model proposed in this research consists of mainly the replacement of P removal processes in the ASM2d+TUD model by the ones included in the Metabolic model (adapted in this thesis). The implication of this extension is the substitution of 11 processes and one microorganism by 55 processes and four microorganisms. Table 15 presents the processes that correspond to each microorganism involved in both models.

Table 15: Summary of processes and microorganisms involved in ASM2d+TUD and Metabolic model

	MODEL		**ASM2d+TUD**	**Metabolic model**			
	Denitrification capacities		$NO_x^- \rightarrow N_2$	**PAOI**: $NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$ **PAOII**: ~~NO_3^-~~ $\rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$ **GB**: ~~NO_3^-~~ $\rightarrow$ ~~NO_2^-~~ $\rightarrow N_2O$ ~~$\rightarrow$~~ N_2 **DGB**: $NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$			
#	**Processes** / **Microorg.**		**PAO**	**PAOI**	**PAOII**	**GB**	**DGB**
1	**Anaerobic VFA uptake**	Acetate	X	X	X	X	X
		Propionate		X	X	X	X
2	**Anaerobic maintenance**		X	X	X	X	X
3	**Aerobic PHA degradation**		X	X	X	X	X
4	**Aerobic glycogen production**		X	X	X	X	X
5	**Aerobic Poly-P formation**		X	X	X		
6	**Aerobic maintenance**		X	X	X	X	X
7	**Anoxic PHA degradation**	on NO3	X	X			X
		on NO2		X	X		X
		on N2O		X	X		X
8	**Anoxic Poly-P formation**	on NO3	X	X			
		on NO2		X	X		
		on N2O		X	X		
9	**Anoxic glycogen production**	on NO3	X	X			X
		on NO2		X	X		X
		on N2O		X	X		X
10	**Anoxic maintenance**	on NO3	X	X			X
		on NO2		X	X		X
		on N2O		X	X		X
11	**Anoxic storage of acetate**		X				
Number of processes			**11**	**19**	**15**	**6**	**15**
TOTAL number of processes			**11**	**55**			

5.1.3 Stoichiometry

The stoichiometric values of the Metabolic Model and the ASM2d+TUD Model were very similar. The stoichiometry of both models are compared in Table 16, which presents the yields used in the matrix of the adapted Metabolic models and the corresponding formulas of the ASM2d+TUD to calculate those yields.

Table 16: Comparison of PAOs yields between the ASM2d+TUD and the Metabolic Model

ANAEROBIC PROCESSES		Units	Adapted Metabolic Model	ASM2d+TUD model	Formula in ASM2d+TUD model
$Y_{Ac\_PHA,PAO}$	PHA stored per Ac taken up by PAO under anaerobic conditions	g COD/g COD	1.50	1.5	$Y_{Ac\_PHA,PAO,An}$
$Y_{Pr\_PHA,PAO}$	PHA stored per Pr taken up by PAO under anaerobic conditions	g COD/g COD	1.29		
$Y_{Gly\_Ac,PAO}$	Glycogen consumed per Ac taken up by PAO under anaerobic conditions	g COD/g COD	0.50	0.5	$-(1-Y_{Ac\_PHA,PAO,An})$
$Y_{Gly\_Pr,PAO}$	Glycogen consumed per Pr taken up by PAO under anaerobic conditions	g COD/g COD	0.29		
$Y_{PO4\_Ac,PAO}$	Poly-P consumed per Ac taken up by PAO under anaerobic conditions	g P/g COD	0.39	0.35	$Y_{PP\_PHA,PAO,An}$
$Y_{PO4\_Pr,PAO}$	Poly-P consumed per Pr taken up by PAO under anaerobic conditions	g P/g COD	0.25		
AEROBIC PROCESSES					
$Y_{O2\_PHA,PAO}$	Oxygen consumed per PHA degraded for PAO biomass synthesis	g O2/g COD	0.25	0.28	$1-1/Y_{PHA\_PAO,Ox}$
$Y_{O2\_Gly,PAO}$	Oxygen not consumed per PHA degraded due to glycogen formation	g O2/g COD	0.11	0.10	$1/Y_{PAO\_PP,Ox}$
$Y_{O2\_PP,PAO}$	Oxygen consumed per PHA degraded due to PP formation	g O2/g P	0.24	0.23	$1/Y_{PAO\_PP,Ox}$
$Y_{PHA\_X,PAO,Ox}$	Maximum PAO biomass production per PHA degraded under aerobic conditions	g COD/g COD	0.75	0.72	$1/Y_{PHA\_PAO,Ox}$
$Y_{X\_Gly,PAO,Ox}$	PAO biomass not produced due to Gly formation under aerobic conditions	g COD/g COD	0.89	0.90	$1/Y_{PAO\_Gly,Ox}$
$Y_{X\_PP\_PAO\_Ox}$	PAO biomass not produced due to PP formation under aerobic conditions	g COD/g P	0.24	0.23	$1/Y_{PAO\_PP,Ox}$
ANOXIC PROCESSES					
$Y_{NO3\_PHA,PAO}$*	Nitrate consumed per PHA degraded for PAO biomass synthesis (considering $Y_{NOx\_PHA,PAO}$ and $Y_{N2O\_PHA,PAO}$)	g N/g COD	0.14	0.15	$(1-1/Y_{PHA\_PAO,Ax})/i_{NOx,N2}$
$Y_{NO3\_Gly,PAO}$*	Nitrate not consumed per PHA degraded due to glycogen formation (considering $Y_{NOx\_Gly,PAO}$ and $Y_{N2O\_Gly,PAO}$)	g N/g COD	0.07	0.05	$-(1/Y_{PAO\_Gly,Ax}-1)/i_{NOx,N2}$
$Y_{NO3\_PP,PAO}$*	Nitrate consumed per PHA degraded due to PP formation (considering $Y_{NOx\_PP,PAO}$ and $Y_{N2O\_PP,PAO}$)	g N/g COD	0.12	0.12	$(1/Y_{PAO\_PP,Ax})/i_{NOx,N2}$
$Y_{PHA\_X,PAO,Ax}$	Maximum PAO biomass production per PHA degraded under anoxic conditions	g COD/g COD	0.61	0.58	$1/Y_{PHA\_PAO,Ax}$
$Y_{X\_Gly,PAO,Ax}$	PAO biomass not produced due to Gly formation under anoxic conditions	g COD/g COD	0.81	0.85	$1/Y_{PAO\_Gly,Ax}$
$Y_{X\_PP,PAO,Ax}$	PAO biomass not produced due to PP formation under anoxic conditions	g COD/g P	0.35	0.33	$1/Y_{PAO\_PP,Ax}$
ANOXIC PROCESSES EXISTED ONLY IN ASM2d+TUD MODEL* **					
$Y_{Ac\_PHA,PAO,Ax}$	PHA stored per Ac uptaken under anoxic conditions	g COD/g COD	n.c	0.71	$Y_{Ac\_PHA,PAO,An}$
$Y_{PO4\_Ac,PAO,Ax}$	Phosphate released per Ac uptaken under anoxic conditions	g P/g COD	n.c	0.23	$Y_{PP\_PHA,PAO,An}$
$Y_{NO3\_Ac,PAO,Ax}$	Nitrate consumed per Ac uptaken under anoxic conditions	g N/g COD	n.c	0.10	$-(1-Y_{Ac\_PHA,PAO,Ax})/i_{NOx,N2}$

*Adjusted values considering the multistep variables denitrification process and assuming no accumulation.

**The yields corresponding to nutrients and alkalinity consumption are not presented because those depend on the other stoichiometric values and the composition of the biomass assumed.
*** The values of the stoichiometry of the anoxic uptake of VFA processes are reasonable with the approach used in the Metabolic model. The PHA stored per Ac uptaken under anoxic conditions ($Y_{Ac\_PHA,PAO,Ax}$= 0.71 g COD/g COD) is less than the one exited under anaerobic conditions ($Y_{Ac\_PHA,PAO}$= 1.5 g COD/g COD) because while PAOII is storing PHA, PAOI is degrading it to form biomass. Likewise, the phosphate released per Ac uptaken under anoxic conditions ($Y_{PO4\_Ac,PAO,Ax}$ = 0.23 g P/g COD) is less than under anaerobic conditions ($Y_{PO4\_Ac,PAO,Ax}$ = 0.35 g P/g COD) because while PAOII is releasing PO4, PAOI is taking-up of it. Nevertheless, since all the acetate stored as PHA would be oxidized by NO3 under anoxic conditions, the nitrate consumed per Ac uptaken under anoxic conditions ($Y_{NO3\_Ac,PAO,Ax}$=0.10 g N/g Ac-COD) is the same as the nitrate consumed per PHA degradated under anoxic conditions ($Y_{NO3\_PHA,PAO}$= 0.15 g N/g PHA-COD) if the conversion is made (0.15 g N/g PHA-COD *1.5 g PHA-COD /g Ac-COD = 0.10 g N/g Ac-COD)

The only values in the stoichiometry that were changed in the Metabolic model corresponded to the aerobic and anoxic maintenance processes. Table 17 shows that the original formula used in the Metabolic model resulted in values up to 12 times smaller than the ones needed to close the COD balance. By using the corrected formula proposed in this thesis, the model not only closed the COD balance but also resulted in the same values like those ones reported in the ASM2d+TUD model.

Table 17: Comparison of maintenance yields of ASM2d+TUD and Metabolic Model

Notation	Description	Units	Metabolic Model (A. Oehmen *et al.*, 2010)		ASM2d+TUD (S. C. Meijer, 2004)	Formula in ASM2d+TUD (S. C. Meijer, 2004)
			Original	Corrected		
m_{NO3}*	Nitrogen requirements for maintenance per biomass concentration (considering m_{NOx} and m_{N2O})	g N/g COD	0.03	0.35	0.35	$-1/i_{NOx,N2}$
m_{Ox}	Oxygen requirements for maintenance per biomass concentration	g O2/g COD	0.86	1	1	-1

*Adjusted values considering the multistep variables denitrification process and assuming no accumulation.

The Tables showed above suggest that the stoichiometry of both models would give almost the same results when acetate is the main component of the VFA and also, when there is no accumulation of intermediates during denitrification. Otherwise, the additional yields included in the Metabolic model for propionate uptake and yields related with intermediates like NO_2^- and N_2O would play a major role.

Worthwhile to mention that for the calibration of the ASM2d+TUD model, the inert solids in the influent and some kinetic values are adjusted but not the stoichiometric values. So the stoichiometry is assumed to be fixed and can be applied for different case studies without any changes.

Based on the stability of the stoichiometry, the yields of the Extended model proposed in this thesis were introduced in the model as input data. Any formula to calculate them was

considered except in cases where pH affects the value. The advantage is that the simulation is simpler and faster.

5.1.4 Maximum storage compounds

The following table shows the values of maximum storage of PHA, glycogen and PP in both models. In the ASM2d+TUD model, a maximum limit for PHA content in the biomass was not considered, the maximum glycogen level is almost double and the maximum PP content is 34% higher compared to the Metabolic model.

Table 18: Comparison of maximum storage compounds between ASM2d+TUD and Metabolic Model

Notation	Description	Units	Metabolic Model (A. Oehmen *et al.*, 2010)		ASM2d+TUD (S. C. Meijer, 2004)	
			Value	Reference	Value	Reference
$f_{PHA,Max}$	Maximum PHA content per PAO or GAO concentration	g COD/g COD	1	(Bengtsson, 2009, A. Oehmen *et al.*, 2010)	-	-
$f_{PAO\_Gly,Max}$	Maximum glycogen content per PAO or GAO biomass concentration	g COD/g COD	0.24	(Smolders *et al.*, 1995)	0.5	(Brdjanovic, Logemann, *et al.*, 1998)
$f_{PAO\_PP,Max}$	Maximum Poly-P content per PAO biomass concentration	g P/g COD	0.26	(Wentzel *et al.*, 1989)	0.35	(Wentzel *et al.*, 1989)

5.1.5 Kinetics

- **Significant differences of kinetics values and half-saturation constants.**

The kinetics of the models presented important differences between them, especially regarding the values of the maximum rates and half saturation constants (K) (see Table 19 and 17 respectively). It is common to find these variations when comparing different models, but it is not possible to measure the impact that these differences would have in the results. This is because the process rate is the result of the maximum rate multiplied by the corresponding Monod equations with different K values. Additionally, there are several switch on/off functions that ideally should not modify the process rate but in reality the multiplication of all these factors may have an impact on the final process rate.

Table 19: Comparison of kinetics between ASM2d+TUD and Metabolic Model

Notation in this model	ANAEROBIC PROCESSES		This model	Meijer	Notation in Meijer model
$q_{PAOI,Ac\_PHA,20}$	Maximum acetate uptake rate for PAOI at 20°C	g COD/g COD/d	**2.1**		
$q_{PAOI,Pr\_PHA,20}$	Maximum propionate uptake rate for PAOI at 20°C	g COD/g COD/d	**2.5**		$q_{PAO,Ac\_PHA,An}$
$q_{PAOII,Ac\_PHA,20}$	Maximum acetate uptake rate for PAOII at 20°C	g COD/g COD/d	**4.3**		
$q_{PAOII,Pr\_PHA,20}$	Maximum propionate uptake rate for PAOII at 20°C	g COD/g COD/d	**5.0**	8	
$m_{PAO,PP,An,20}$	ATP necessary for anaerobic maintenance purposes of PAOs at 20°C	g P/g COD/d	**0.05**	0.05	$m_{PAO,An}$
	AEROBIC PROCESSES				
$q_{PAO,PHA\_Ac,Ox,20}$	PHA_Ac degradation rate by PAO under aerobic conditions	g COD/g COD/d	**19.9**		q_{PHA_PAO}
$q_{PAO,PHA\_Pr,Ox,20}$	PHA_Pr degradation rate by PAO under aerobic conditions	g COD/g COD/d	**8.2**	5.51	
$q_{PAO,Gly\_Ac,Ox,20}$	Glycogen_Ac formation by PAO under aerobic conditions	g COD/g COD/d	**0.32**		q_{Gly}
$q_{PAO,Gly\_Pr,Ox,20}$	Glycogen_Pr formation by PAO under aerobic conditions	g COD/g COD/d	**0.16**	0.93	
$q_{PAO,PO4\_PP\_Ac,Ox,20}$	Aerobic P-uptake rate by PAO, depending on acetate in the influent (Std. cond)	g P/g COD/d	**0.41**		$q_{PAO,PO4\_PP}$
$q_{PAO,PO4\_PP\_Pr,Ox,20}$	Aerobic P-uptake rate by PAO, depending on propionate in the influent (Std. cond)	g P/g COD/d	**0.04**	0.1	
$m_{PAO\_PHA,Ox,20}$	Oxygen necessary for aerobic maintenance purposes of PAO at 20°C	g O2/g COD/d	**0.09**	0.06	$m_{PAO,Ox}$
	ANOXIC PROCESSES				
$q_{PAO,PHA\_Ac,Ax,20}$	PHA_Ac degradation rate by PAO under anoxic conditions	g COD/g COD/d	**7.5**		$q_{PHA\_PAO*nqPAO}$
$q_{PAO,PHA\_Pr,Ax,20}$	PHA_Pr degradation rate by PAO under anoxic conditions	g COD/g COD/d	**1.2**	4.41	
$q_{PAO,Gly\_Ac,Ax,20}$	Glycogen_Ac formation by PAO under anoxic conditions	g COD/g COD/d	**0.43**		$q_{Gly*nqPAO}$
$q_{PAO,Gly\_Pr,Ax,20}$	Glycogen_Pr formation by PAO under anoxic conditions	g COD/g COD/d	**0.05**	0.74	
$q_{PAO,PO4\_PP\_Ac,Ax,20}$	Anoxic P-uptake rate by PAO, depending on acetate in the influent (Std. cond)	g P/g COD/d	**0.10**		$q_{PAO,PO4\_PP*nqPAO}$
$q_{PAO,PO4\_PP\_Pr,Ax,20}$	Anoxic P-uptake rate by PAO, depending on propionate in the influent (Std. cond)	g P/g COD/d	**0.02**	0.08	
$m_{PAO\_PHA,Ax,20}$	Nitrogen necessary for aerobic maintenance purposes of PAO at 20oC	g N/g COD/d	**0.09**	0.09	$m_{PAO,Ax}$

$_{nqPAO}$ **= 0.8**

Table 20: Comparison of constant for half sturation and switch function equations in the model.PAO processes.

Notation	Coefficient for	Units	Metabolic Model (A. Oehmen *et al.*, 2010)		ASM2d+TUD (S. C. Meijer, 2004)	
			Value	Reference	Value	Reference
K_{S_Ac}	Acetate	$gCOD/m^3$	0.03	Switch	4	(Mogens Henze *et al.*, 1999)
K_{S_Pr}	Propionate	$gCOD/m^3$	0.04	Switch	--	--
K_{NO3}	Nitrate	gN/m^3	0.01	Switch	0.5	(Mogens Henze *et al.*, 1999)
K_{NO2}	Nitrite	gN/m^3	0.01	Switch	--	--
K_{N2O}	Nitrous oxide	gN/m^3	0.01	Switch	--	--
K_{O2}	Oxygen	$gO2/m^3$	0.32	Switch	0.2	(Mogens Henze *et al.*, 1999)
K_{fPHA}	PHA fraction	gCOD /gCOD	0.01	Switch	0.2	(S. C. Meijer, 2004)
$K_{PO4,GAO}$	Phosphate as nutrient	gP/m^3	0.32	Switch	0.02	(S. C. Meijer, 2004)
$K_{PO4,PAO}$	Phosphate for PAOs	gP/m^3	3.20	Switch	1	(S. C. Meijer, 2004)
K_{NHx}	Ammonium nitrogen	gN/m^3	--	--	0.05	(S. C. Meijer, 2004)
K_{Alk}	Alkalinity	$molHCO_3/m^3$	---	---	0.01	Switch
g_{PP}	Saturation reduction factor for PP formation	---	---	---	0.22	(Murnleitner *et al.*, 1997)
Strictly to avoid indetermination						
K_{VFA}	Volatile Fatty Acids	$gCOD/m^3$	--	--	--	--
$K_{NO3,NO2}$	Preference for nitrate over nitrite	gN/m^3	0.001	Switch	--	--
$K_{NO2,N2O}$	Preference for nitrite over nitrous oxide	gN/m^3	--	--	--	--
K_{PP}	Poly-P in PAOs	gP/m^3	0.31	Switch	0.01	Switch
K_{Gly}	Glycogen in PAOs and GAOs	$gCOD/m^3$	0.32	Switch	0.01	Switch
K_{PHA}	PHA in PAOs and GAOs	$gCOD/m^3$	37.17	Switch	0.01	Switch
$K_{f\_PHA\_max}$	Difference between $f_{PHA,Max}$ and f_{PHA} in PAOs and GAOs	gCOD/gCOD	0.05	Switch	---	---
$K_{f\_PP\_max}$	Difference between $f_{PP,Max}$ and f_{PP} in PAOs	gP/gCOD	0.009	Switch	0.01	Switch
$K_{f\_Gly\_max}$	Difference between $f_{Gly,Max}$ and f_{Gly} in PAOs and GAOs	gCOD/gCOD	0.009	Switch	0.01	Switch

Important to know is that all the K values in the Metabolic model are very small and are not based in half-saturation values reported in the literature. Their application was merely to 'stop' the processes whenever the limiting compound becomes exhausted. This means that in this model several process rates tend to be constants that turn on and off depending on the conditions in the reactor. On the other hand, the ASM2d+TUD model has several K values based on literature which would regulate the process rates, particularly when the concentrations are small. For this reason, the role of the differences in K values would be more noticeable when the concentration approaches the K values.

- **Reduction factor for denitrification processes (n_{qPAO})**

The ASM2d+TUD model assumes only one PAO population with certain kinetic activity under aerobic conditions and the same activity multiplied by a reduction factor (n_{qPAO}) to express the activity under anoxic conditions. The factor n_{qPAO} tends to vary significantly, depending on the case of study, and its range is typically between 0.5 and 0.8 (Brdjanovic *et al.*, 2000, Murnleitner *et al.*, 1997, van Veldhuizen *et al.*, 1999).

On the other hand, in the metabolic model the kinetics of the microorganisms under aerobic and anoxic conditions are independent between each other and do not use a reduction factor. As a consequence, if the metabolic model is applied there is no need to calibrate a reduction factor that may change significantly from case to case, and the denitrifying-phosphorus removal capacity of the process will be a consequence and a reflection of the occurrence of bacterial communities capable of denitrifying (e.g. PAOI). The only process in the metabolic model that considers any correction of the process rate due to denitrification processes is the anaerobic VFA uptake (see Appendix A.5)

- **Anaerobic VFA uptake**

The ASM2d+TUD model has a single process for anaerobic VFA uptake, whereas the Metabolic Model splits this process into the anaerobic uptake of acetate and propionate. Additionally, the metabolic model included two switching functions, (a) one to make the kinetic proportional to the abundance of each kind of VFA, preventing the duplication of the rate, and (b) another to stop the process when the PHA has accumulated up to the maximum level.

a) $$\frac{S_{Ac}}{(S_{Ac}+S_{\Pr})+K_{VFA}}, \frac{S_{\Pr}}{(S_{Ac}+S_{\Pr})+K_{VFA}}$$

b) $$\frac{f_{PHA,\max}-f_{PAO,PHA}}{f_{PHA,\max}-f_{PAO,PHA}+K_{fPHA}}$$

- **Factor $f_{PHA}^{2/3}$ for PHA degradation and glycogen production rates**

The Metabolic model included an additional factor in the processes of PHA degradation and glycogen production under aerobic and anoxic conditions. This factor was suggested in order to express the relation betwee PHA degradation and the area-volume ratio of this compound (Murnleitner et al., 1997):

$$f_{PAO,PHA}^{2/3}, f_{GAO,PHA}^{2/3}$$

- **Cascade multi-steps reactions**

The Metabolic model considered denitrification coupled with P removal as a 3- step process (NO_3^- --> NO_2^- --> N_2O) whereas in the ASM2d+TUD model it is assumed as a 1-step process. Additionally, the multistep process was simulated as a cascade reaction in which the preference of the microorganisms to use the electron acceptor with higher oxidation rate was expressed as:

$$\frac{S_{NO2}}{(S_{NO2}+S_{NO3})+K_{NO3,NO2}}, \frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_{NO2,N2O}}$$

- **Inhibitory effect of free nitrous acid S_{HNO2}**

The Metabolic model included the inhibitory effect of S_{HNO2} by the addition of an exponential function in processes for PHA degradation and PP formation of PAOs in order to consider its impact in N2O reduction (Zhou *et al*., 2007):

a) $e^{-\frac{170}{14}\cdot S_{HNO2}}$:For anoxic PP formation by PAOs (on NO_2^-, NO_3^- and N_2O)

b) $e^{-\frac{760}{14}\cdot S_{HNO2}}$: For PHA degradation by PAOs (on N_2O)

- **Use of a reduction factor for K affinity in PP formation**

The ASM2d+TUD model introduced a reduction factor (n_{KO2} in standard notation or g_{PP} in the original notation) in order to simulate the PP formation under aerobic and anoxic conditions by correcting the $K_{O2,PAO}$ and $K_{NOx,PAO}$ respectively. This factor, which needs to be calibrated to fit the anoxic PP formation, can vary from 0.1 to 0.25 (Brdjanovic *et al.*, 2000, Murnleitner *et al.*, 1997, van Veldhuizen *et al.*, 1999). The addition of n_{KO2} was not considered in the Metabolic model because, as mentioned earlier, the K values in that model are used to activate the switch on/off functions.

5.2 PAO-GAO competition

In the ASM2d+TUD model (Meijer, 2004), the presence of GAOs was suggested when the anaerobic VFA take up rate was higher compared to the respective aerobic and anoxic

PP formation. As a consequence, the correction of the model due to the presence of GAOs was made up to certain limit, by changing the mentioned rates. Nevertheless, the approach used in the Extended model (proposed in this thesis) allows to simulate the PAO-GAO competition.

In this subchapter, the PAO-GAO competition is analyzed firstly under standard conditions. Then, using as a reference the PAO-culture tested previously using the Extended model, some input data in the simulation was modified in order to analyse how PAO and GAOs populations change after three times the sludge retention time.

The following conditions were compared:

- Change of pH 6, 7 and 7.5
- Change of temperature 10 ^{o}C, 20 ^{o}C and 30 ^{o}C
- Change of Ac/VFA composition 0%, 60% and 100%

The outcomes of the simulations and the analysis of the variables that impact PAO-GAO competition are analyzed below.

5.2.1 PAO-GAO competition under standard conditions

When the yield values of PAOs and GAOs reported in the Metabolic model (Oehmen *et al.*, 2010) are compared (see Table 21), it is noticeable that both microorganisms have similar stoichiometries except during the anaerobic phase. GAOs tend to consume more glycogen during anaerobic VFA uptake ($Y_{Gly\_Ac,GAO}$ and $Y_{Gly\_Pr,GAO}$) because glycogen is their sole source of energy, whereas PAOs obtain energy from glycogen and also from PP. Considering that the PHA stored is the sum of acetate taken up and the glycogen consumed, the PHA stored per VFA taken up is higher in GAOs than PAOs ($Y_{Ac\_PHA,GAO}$ and $Y_{Pr\_PHA,GAO}$)

Table 21: Comparison of stoichiometric rates for PAOs and GAOs

ANAEROBIC PROCESSES		**Units**	**Value for GAOs**	**Value for PAOs**
$Y_{Ac\_PHA,GAO}$	PHA stored per Ac taken up by GAOs under anaerobic conditions	g COD/g COD	**2.17**	**1.50**
$Y_{Pr\_PHA,GAO}$	PHA stored per Pr taken up by GB under anaerobic conditions	g COD/g COD	**1.51**	**1.29**
$Y_{Gly\_Ac,GAO}$	Glycogen consumed per Ac taken up by GAOs under anaerobic conditions	g COD/g COD	**1.12**	**0.50**
$Y_{Gly\_Pr,GAO}$	Glycogen consumed per Pr taken up by GAOs under anaerobic conditions	g COD/g COD	**0.57**	**0.29**
AEROBIC PROCESSES		**Units**	**Value for GAOs**	**Value for PAOs**
$Y_{O2\_PHA,GAO}$	Oxygen consumed per PHA degraded for GAOs biomass synthesis	g O2/g COD	0.24	0.25
$Y_{O2\_Gly,GAO}$	Oxygen not consumed per PHA degraded due to glycogen formation	g O2/g COD	0.11	0.11
$Y_{PHA\_X,GAO,Ox}$	Maximum GAO biomass production per PHA degraded under aerobic conditions	g COD/g COD	0.76	0.75
$Y_{X\_Gly,GAO,Ox}$	GB biomass not produced due to Gly formation under aerobic conditions	g COD/g COD	0.89	0.89
ANOXIC PROCESSES		**Units**	**Value for GAOs**	**Value for PAOs**
$Y_{NOx\_PHA,DGB}$	Nitrate/Nitrite consumed per PHA degraded for DGB biomass synthesis	g N/g COD	0.34	0.34
$Y_{NOx\_Gly,DGB}$	Nitrate/ Nitrite not consumed per PHA degraded due to glycogen formation	g N/g COD	0.17	0.17
$Y_{N2O\_PHA,DGB}$	Nitrous oxide consumed per PHA degraded for DGB biomass synthesis	g N/g COD	0.67	0.68
$Y_{N2O\_Gly,DGB}$	Nitrous oxide not consumed per PHA degraded due to glycogen formation	g N/g COD	0.34	0.33
$Y_{PHA\_X,DGB,Ax}$	Maximum DGB biomass production per PHA degraded under anoxic conditions	g COD/g COD	0.62	0.61
$Y_{X\_Gly,DGB,Ax}$	DGB biomass not produced due to Gly formation under anoxic conditions	g COD/g COD	0.81	0.81

The Table 22 compares the kinetic of PAOs and GAOs according to the values reported in the Metabolic model (Oehmen *et al.*, 2010). There is a significant difference in the anaerobic propionate uptake rate of both microorganisms. GAOs are hardly able to use propionate as a carbon source (see $q_{GB,Pr\_PHA,20}$ and $q_{DGB,Pr\_PHA,20}$), whereas PAOs cannot only use propionate but also tend to assimilate it faster than acetate. Moreover, the anaerobic VFA uptake in GAOs is sensible to pH, whereas all kinetics in PAOs are constants (see Figure 17).

As a result, the pH and the composition of acetate and propionate would impact considerably the efficiency in which PAOs and GAOs can assimilate VFA during the anaerobic phase, and so can proliferate during the subsequent anoxic/aerobic phases.

Another important difference in the kinetics of both microorganisms is the glycogen formation rate, especially under aerobic conditions in which the GAOs rate ($q_{GAO,Gly,Ox,20}$)

is aproximately 20 times higher than PAOs rate. The latter would have an important effect in the growth of the microorganisms.

Table 22: Comparison of kinetic rates for PAOs and GAOs

ANAEROBIC PROCESSES		**Units**	**Value**	**Value for PAO**
$q_{GB,Ac\_PHA,20}$	Maximum acetate uptake rate for GB at 20°C	g COD/g COD/d	4.3*	PAOI=2.1 PAOII= 4.3
$q_{DGB,Ac\_PHA,20}$	Maximum acetate uptake rate for DGB at 20°C	g COD/g COD/d	2.1*	
$q_{GB,Pr\_PHA,20}$	Maximum propionate uptake rate for GB at 20°C	g COD/g COD/d	**0.25***	**PAOI=2.50** **PAOII= 5.0**
$q_{DGB,Pr\_PHA,20}$	Maximum propionate uptake rate for DGB at 20°C	g COD/g COD/d	**0.1***	
$m_{GAO,Gly,An,20}$	Gly necessary for anaerobic maintenance purposes of GB and DGB at 20°C	g COD/g COD/d	0.09	n.c.
AEROBIC PROCESSES		**Units**	**Value**	**Value for PAO**
$q_{GAO,PHA,Ox,20}$	PHA degradation rate by GB / DGB under aerobic conditions	g COD/g COD/d	27.6	**19.9****
$q_{GAO,Gly,Ox,20}$	Glycogen formation by GAO under aerobic conditions	g COD/g COD/d	**6.4**	**0.3****
$m_{GAO\_PHA,Ox,20}$	Oxygen necessary for aerobic maintenance purposes of GB at 20°C	g O2/g COD/d	0.09	0.09
ANOXIC PROCESSES		**Units**	**Value**	**Value for PAO**
$q_{DGB,PHA,Ax,20}$	PHA degradation rate by DGB under anoxic conditions	g COD/g COD/d	10.5	7.5**
$q_{DGB,Gly,Ax,20}$	Glycogen formation by GAO under anoxic conditions	g COD/g COD/d	**0.8**	**0.4****
$m_{DGB,PHA,Ax,20}$	Nitrogen necessary for aerobic maintenance purposes of DGB at 20°C	g N/g COD/d	0.09	0.09

* pH dependant variables. See Table 22 of the Appendix.

**PAOs count with different values for PHA degradations and glycogen storage depending on the source of carbon (acetate and propionate). Nevertheless, the uptake of Pr by GAOs is neglected and the kinetic values depends on acetate uptake. For this reason the kinetic values for GAOs were compared with the kinetic values of PAOs corresponding to acetate.

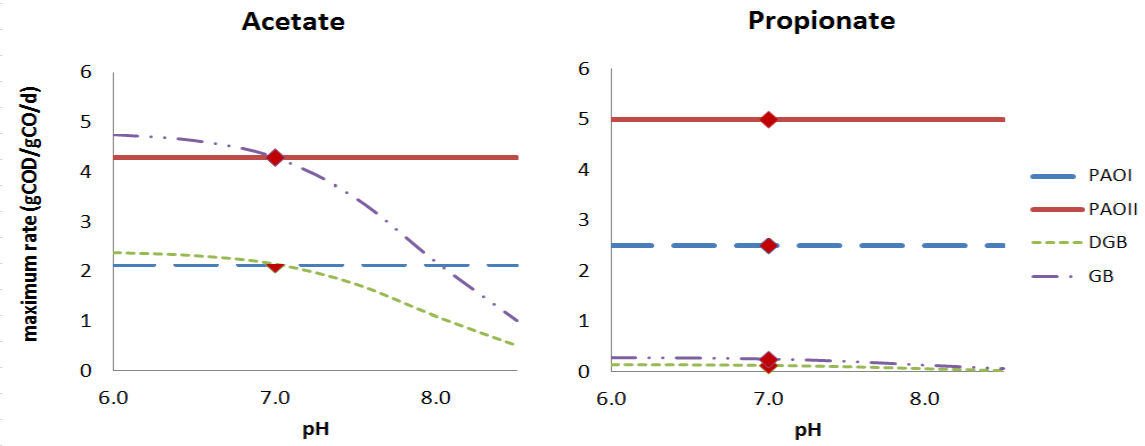


Figure 17: Maximum anaerobic VFA uptake rate of PAOs and GAOs depending of influent composition and pH

The maximum PHA storage compounds is the same for GAOs and PAOs (Oehmen *et al.*, 2010), but the maximum storage of glycogen is 30% higher in GAOs (López-Vázquez *et al.*, 2008). The reason of this difference, as mentioned earlier, is because glycogen is the only source of energy under anaerobic conditions for GAOs, whereas PAOs are able to use glycogen and also PP.

5.2.2 PAO-GAO competition when pH changes

The Figure 18 presents the cycle profile and the occurrence of microbial populations in the PAO-culture when pH changes. As reported in the literature (Lopez-Vazquez, 2009), the simulations showed that the higher the pH, the higher occurrence of PAOs over GAOs (*Competibacter*). As a result, when pH increases the concentration of glycogen decreases and the PP storage increases (reflected in the release of phosphates during anaerobic stage).

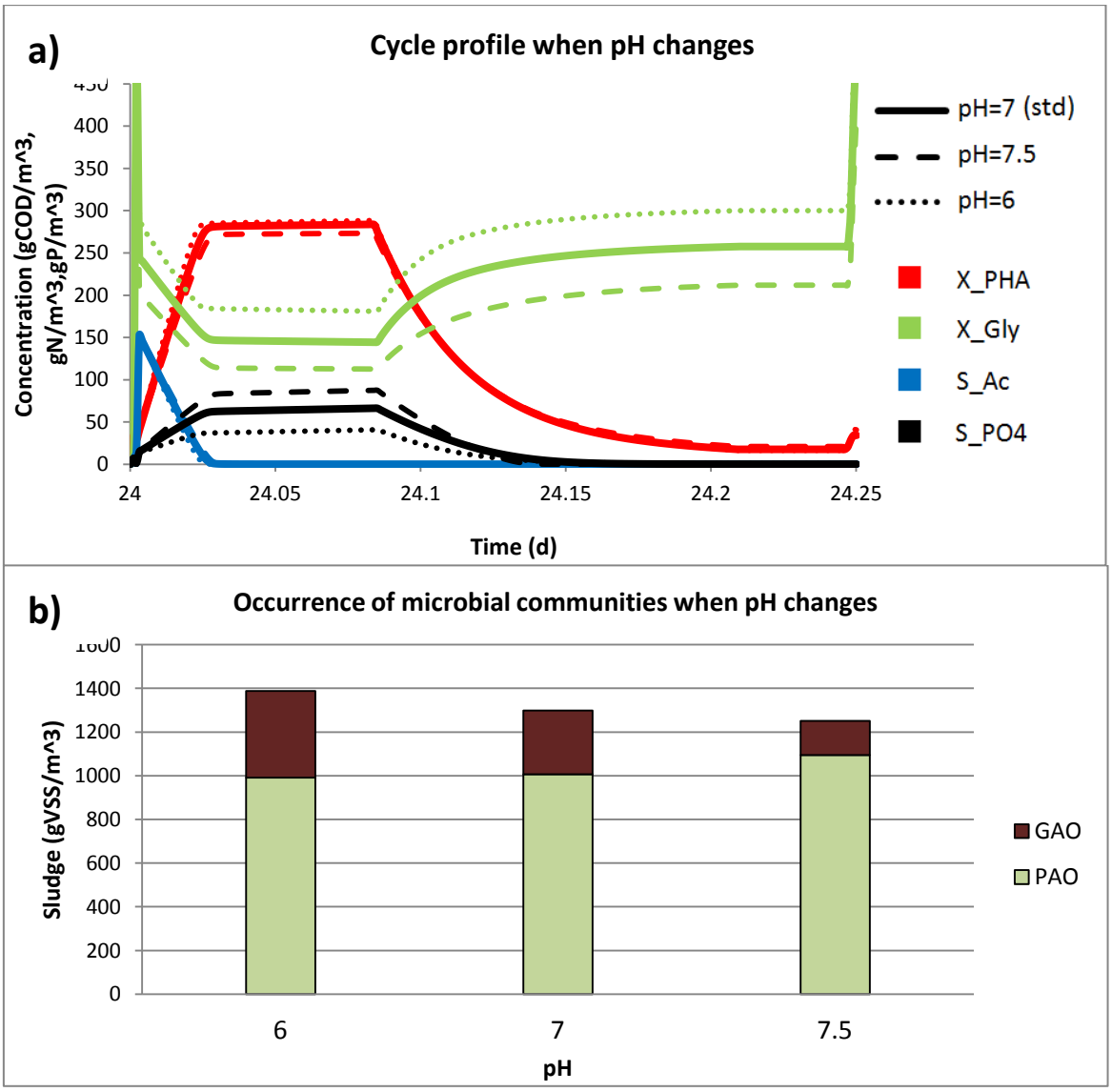


Figure 18: Simulation of PAO-culture when pH changes: a) Cycle profile, b) occurrence of microbial populations.

The pH impacts significantly the anaerobic stoichiometry of PAOs and GAOs and the anaerobic kinetics of GAOs (see Table 22 in the Appendix). These dependencies were incorporated in the Extended model.

5.2.3 PAO-GAO competition when temperature changes

The Figure 19 presents the cycle profile and the occurrence of microbial populations in the PAO-culture when temperature changes. As reported in the literature (Lopez-Vazquez, 2009), the simulations showed that the higher the temperature, the lower the occurrence of PAOs populations due to its psychrophilic metabolism. Consequently at high temperature the remotion of P decreases and the glycogen concentration increases (due to GAOs presence).

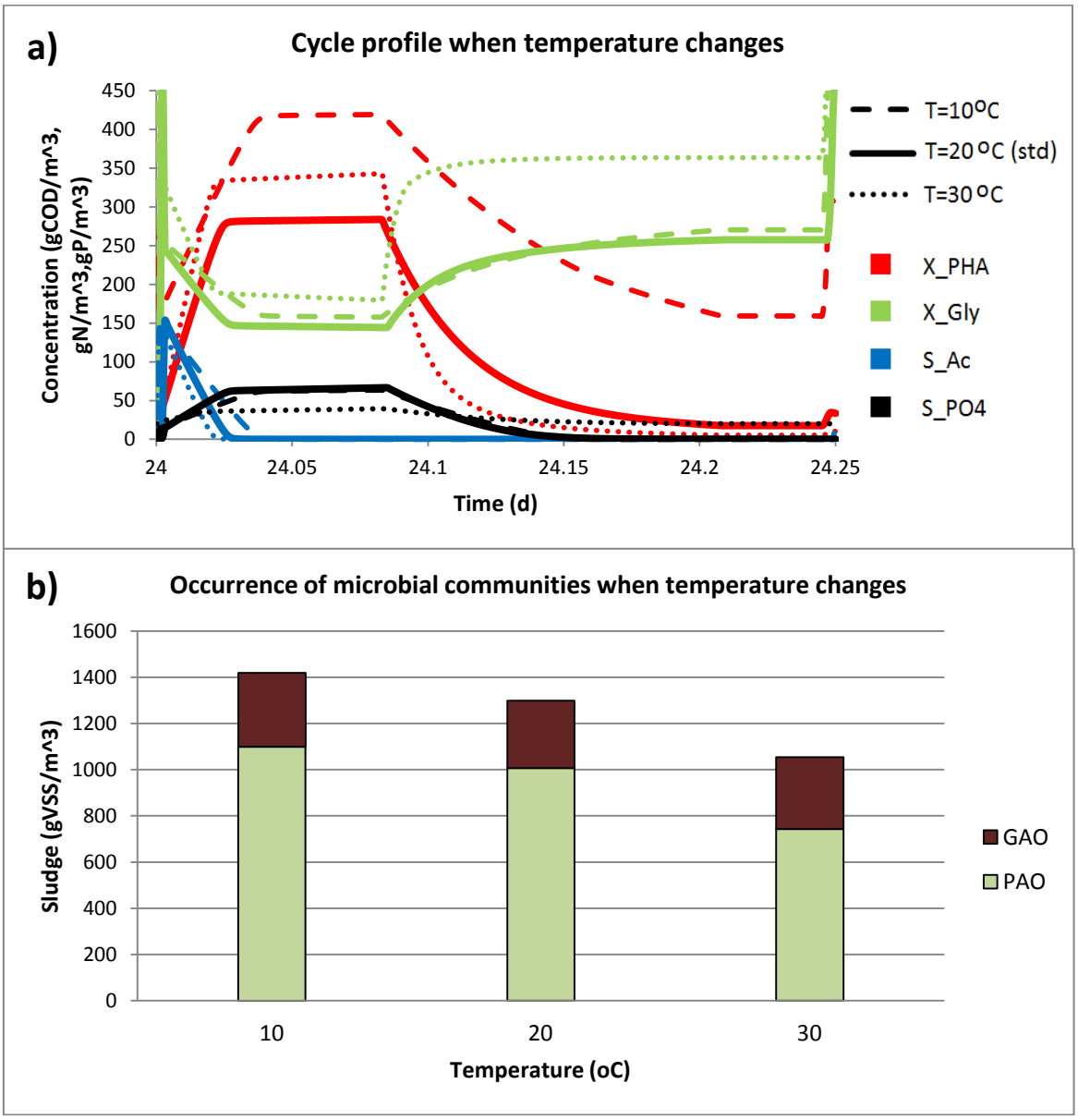


Figure 19: Simulation of PAO-culture when temperature changes: a) Cycle profile, b) occurrence of microbial populations.

5.2.4 PAO-GAO competition when Ac/VFA ratio changes

The Figure 20 presents the cycle profile and the occurrence of microbial populations in the PAO-culture when the compositions of VFA changes. As reported in the literature (Lopez-Vazquez, 2009), the propionate can hardly be taken up by GAOs, so the presence of propionate give a competitive advantage to PAOs (Oehmen *et al.*, 2005, Pijuan *et al.*, 2004). On the contrary, PAOs can take up propionate faster than acetate. For this reason, the higher the concentration of propionate, the higher occurrence of PAOs and the faster the removal of P.

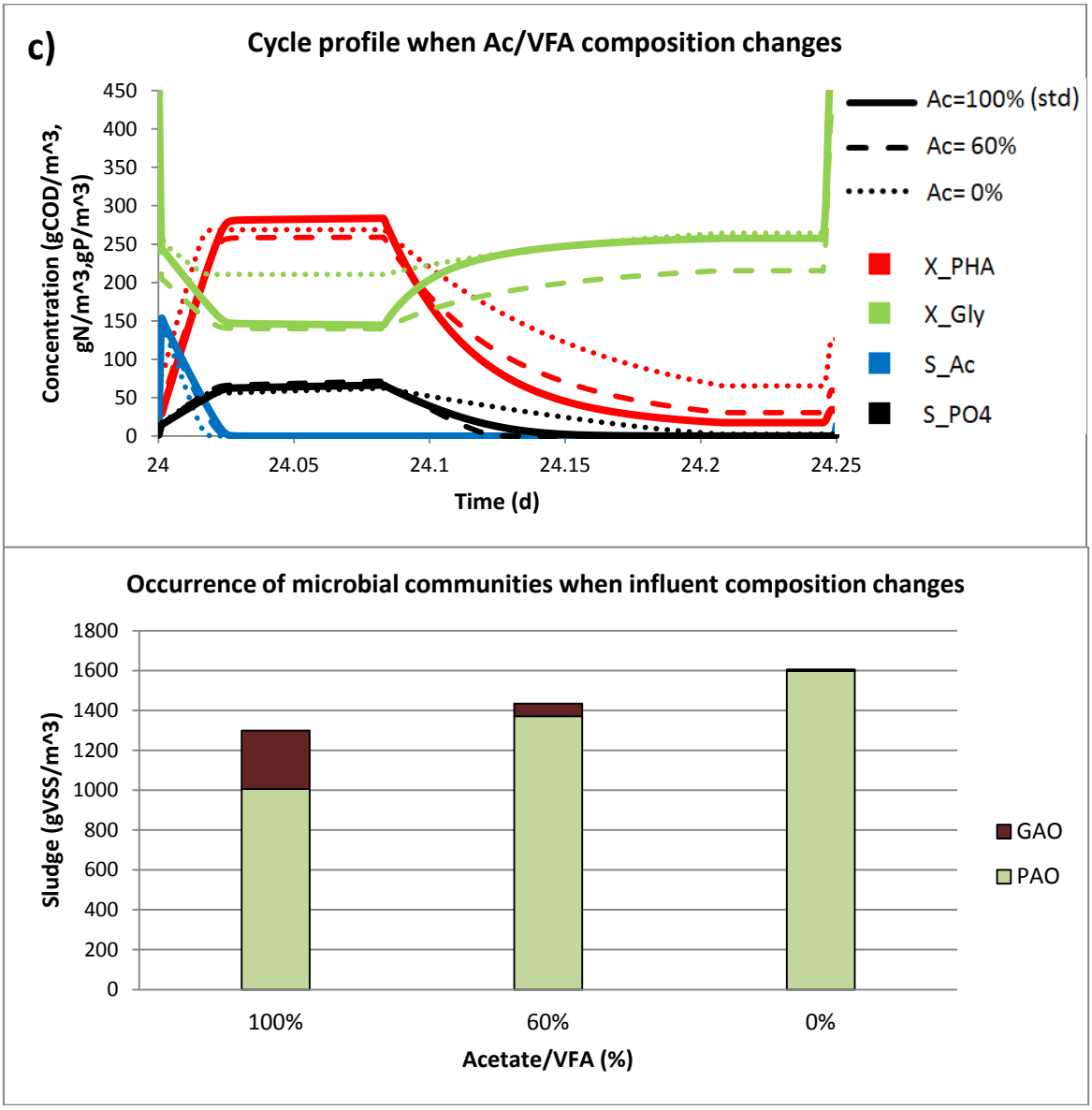


Figure 20: Simulation of PAO-culture when Ac/VFA ratio changes: a) Cycle profile, b) occurrence of microbial populations.

5.3 Boundaries of the model

The Extended model proposed in this thesis has the following boundaries:

5.3.1 Valid for municipal WWTP

This model is valid for typical municipal wastewater, within a range of pH between 6 and 8.5, and temperature between 10 and 30 ^{o}C. The industrial wastewaters may have other inhibitory coumpunds, ions, metales or extreme temperature or pH values that may affect the enzymes functioning of the microorganisms, which is not considered in this model.

5.3.2 pH changes does not affect OHOs and ANOs processes

The impact of pH was included for all the processes involved in PAOs and GAOs communities. Nevertheless, the impact of pH is not included directly in the kinetics and stoichiometry of OHOs and ANOs, if it exists. Nevertheless, the model includes switch on/off functions for alkalinity. The inconvenience is that when alkalinity is a limiting factor, it may affect the process rate, which was neglected in the ASM2d+TUD model (Hauduc, 2010) and so in the Extended model.

5.3.3 No predictions of N2O release into the atmosphere

This model is supposed to be able to detect any accumulation of intermediaries during the multistep nitrification and denitrification processes (still need validation though). However, it does not include the chemical and physical variables necessary to calculate the emissions of N_2O into the atmosphere, which depends on the solubility of this molecule in the water affected by the temperature, pH, k_La, turbulence of the reactor, etc (Davidson & Swank, 1986).

Nevertheless, a model that includes this quantification would still have uncertainties due to the very basic methodologies that exists to estimate methane and nitrous oxide emissions from full-scale wastewater treatment plants (Foley *et al.*, 2010, IPCC, 2006).

5.3.4 Not valid when there is inhibitory accumulation of intermediaries.

As the Metabolic model (Oehmen *et al.*, 2010) did, the proposed model included the inhibition of PHA degradation and PP formation due to accumulation of S_{HNO2}. Nevertheless, in general the NO_2 is a toxic compound for growth and respiration processes in microorganisms (Rowe *et al.*, 1979, Yarbrough *et al.*, 1980) due to its

affinity to the metal iones located in the active centre of enzymes (Wild *et al.*, 1995). This inhibitory effect has been found for heterotrophs microorganisms (Nowak *et al.*, 1995). Therefore, it would be recommendable to include the inhibition effect of the intermediaries in the other communities of the model, if there is a possibility of intermediates accumulation.

5.3.5 Inaccuracy in fermentation and hydrolysis processes

There is still inaccuracy in fermentation and hydrolysis processes in the ASM2d+TUD model (Meijer, 2004). For this reason it is recommend to estimate VFA uptake experimentally and then to calibrate fermentation and hydrolysis processes. Nevertheless, for the further research it would be recommendable to include a better approach to simulate hydrolysis and especially fermentation processes, which has a high impact in P removal systems. Moreover, there are other processes and components that are involved in the fermentation. There are other species of VFA besides acetate and propionate, such as butyrate and valerate. Moreover, the long chains of VFA tend to hydrolyze to shorter molecules and so, the acetate may increase along a fermentation process.

In the Extended model proposed in this thesis, the fermentation only considered a transformation from fermentable COD into acetate and propionate, keeping the same relation Ac/VFA given in the influent. So it was assumed that the fermentation does not change the composition and relation acetate/propionate. Longer chains of VFA were neglected.

This could be not be a good assumption when the anaerobic stage takes place in tanks with long retention times, like in pre-fermentor tanks.

Also, it is valid for certain position of VFA (propoinate 0-25%), if it is higher, there would be alphaproteobacteria (Deflivoccoccus), (Oehmen 2010b)

5.3.6 Simultaneous nitrification-denitrification

In full-scale activated sludge systems, the denitrification is not an on/off process, there is still denitrification when there is low concentration of oxygen (Schulthess & Gujer, 1996). This simultaneous nitrification-denitrification is solved in the ASM2d+TUD model (Meijer, 2004) by adjusting the half saturation constant (K_{O2}) that is shared for nitrification and denitrification processes. Nevertheless, when the Extended model included multi-steps nitrification-denitrification processes, it considered the K_{O2} values proposed in the models of reference (Schulthess & Gujer, 1996, Sin *et al.*, 2008a), in which these constants are independent each other because it was found that oxygen inhibited denitrification enzimes in a different extent. For this reason, the parameters of

nitrificacion and denitrification used in the Extended model require further validation. Furthermore, it is indispensable to develop a new approach to simulated the simultaneous nitrificacion-denitrificacion processes.

5.4 Further research

5.4.1 Calibration and validation of a integrated case study

The different parts of this model need to be calibrated with laboratory and/or full-scale data, including not only the processes of PAOs and GAOs replaced in the ASM+TUD model, but also the incorporated multi-step processes for nitrification and denitrification. Moreover, it order to test the model entirely, it is also necessary to calibrate and validate the complete model using data from an integrated case study, preferably from a full-scale wastewater treatment plant. A full calibration, validation and testing of the model under different scenarios (environmental conditions and influent parameters) would allow to obtain even more valuable results and conclusions regarding the use of this model.

5.4.2 New configuration proposed (modified Johannesburg)

After a general evaluation of the most common configurations for EBPR systems used for mainstreams (3-Stage Modified Bardenpho, Johannesburg, Modified UCT, Biological-chemical phosphorus and nitrogen removal), it is proposed in this thesis to modify the Johannesburg (JHB) configuration in order to get rid of the electron acceptors (O_2 ,NO_3^- and/or NO_2^-) that may remain in sludge in a more efficient way. This will help to avoid the deterioration of EBPR-related systems since the anaerobic stages are not fully anaerobic but anoxic instead (see Figure 21).

In the original JHB configuration, part of the influent is by-passed to a pre-anoxic reactor in which the remaining NOx and oxygen present in the sludge drops before entering into the anaerobic reactor. This may increase the availability of VFA for PAO growth in the anaerobic reactor. Nevertheless, since part of the raw influent is being directed to the pre-anoxic reactor, the load of readily biodegradable organic matter to the system is reduced. Moreover, the pre-denitrification process will hardly exhibit PAOI/PAOII activity during this specific step. The reason is that the sludge is coming from the aerobic stage, so the PAOs do not have reserves of PHA that they can consume during this step.

It is suggested an adjustment of the JHB process to reduce this drawback. The modification proposed consists on directing part of the wastewater stream coming from the anaerobic reactor into the pre-denitrification tank.

The advantages of the modified configuration (called MJHB-IHE in this thesis) would be that the limiting VFAs present in the raw wastewater will not be spent in the pre-denitrifying process, so available substrate for PAOs/DPAOs growth is not reduced. Moreover, part of the PAO/DPAOs would be able to grow in the pre-denitrification reactor because the effluent of the anaerobic stage contains microorganisms that are full of PHA reserves.

Additionally, if this process is compared with the modified UCT configuration (MUCT) in which the effluent of the anaerobic reactor is also used to denitrify the sludge, it is clearly noticeable that the pumping requirements would be significantly lower in the modified JHB proposed in this thesis than in the MUCT. The reason is that the systems with pre-denitrification in the mainstream (not in a separated side tank like JHB), the sludge recycled is highly diluted by the influent and so, it requires a higher sludge recycle flow to the anaerobic tank.

And finally, if the proposed MJHB-IHE configuration is compared to the Biological-chemical phosphorus and nitrogen removal (BCFS) configuration, it can be seen that the modified JHB configuration is simpler and with less pumping requirements.

The drawbacks of this process are that the pre-denitrification reactor would be larger than in JHB configuration. This is because during the anaerobic process the more easily biodegradable matter has already been consumed. Another disadvantage is that the PAOI/PAOII growth will not be enhanced as much as in MUCT and BCFS process during the pre-denitrification step. The reason is that the concentration of PAOI/PAOII that contain high concentrations of PHA entering into this reactor will be significantly lower, taking into account that only a derivation of the anaerobic effluent will enter into this reactor. Moreover, the modified JHB would not be as flexible as a BCFS process to remove N during pick flows and/or pick loads.

For the reasons mentioned above, it is recommendable for further research to compare the MJHB-IHE with the other configurations. Under certain conditions, the configuration proposed could have lower operational costs compared to existing process configurations due to its simplicity and enhancement of denitrifying PAO activity. The first one would reduce pumping requirements; while the latter would help to reduce the sludge production and oxygen requirements.

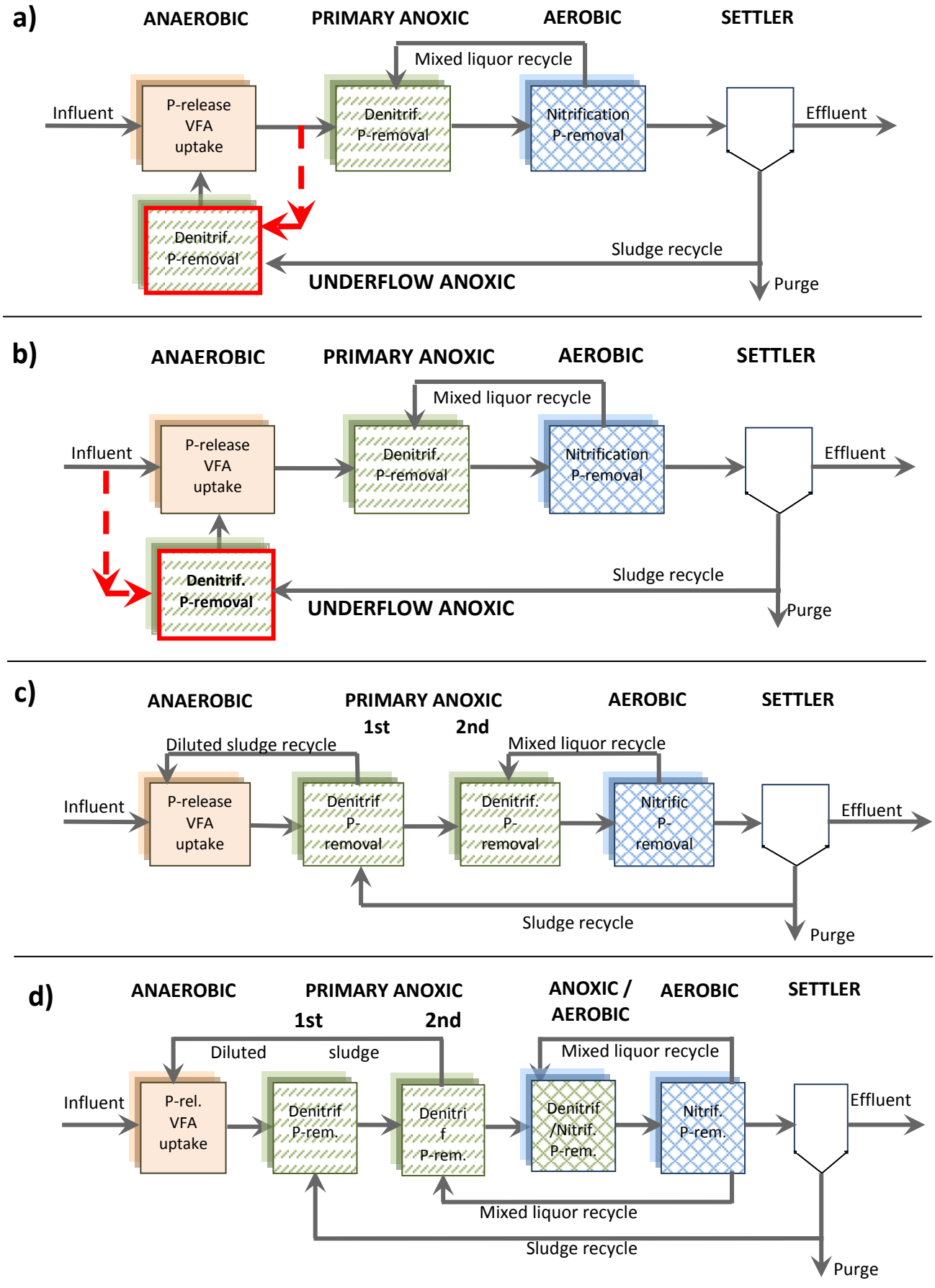


Figure 21: Process configuration of: MJHB-IHE (proposed in this thesis), b) Johannesburg (original), c) Modified UCT, and d) BCFS

6 Conclusions

- The 'Extended ASM2d+TUD model' proposed in this thesis incorporated the activity of Ordinary Heterotrophic Organisms, two subgroups of Phosphorus Accumulating Organisms (PAOI and PAOII), two subgroups of PAOs' competitors that may affect P-removal efficiencies (*Competibacter* and Denitrifying *Competibacter*) and two subgroups involved in the nitrification processes (Ammonia oxidizing organisms and Nitrite oxidizing organisms).

  In order to build the proposed model, the ASM2d+TUD model (Meijer, 2004) and the Metabolic model (Oehmen *et al.*, 2010) required to be adapted, so they have compatibility before joining them in a single model. **The balance of COD, N, P and charges probed that the stoichiometry of new models was consistent**.

- A sensitivity analysis was carried out in order to measure the impact of fluctuations in pH and influent VFA composition (within a range that is characteristic of municipal wastewater) on the parameters of the biological P removal processes. The results showed that when the pH changes between a value of 6 and 8.5, the stoichiometric and kinetic values of the anaerobic processes of PAOs and GAOs can change significantly (and non-linearly) compared to the value when pH is neutral (up to 90% of difference). On the other hand, when the acetate concentration fluctuates between 50% and 75% from the total VFA, large number of parameters corresponding to aerobic and anoxic processes are affected, but the impact is relatively low (<4% compared to the average value).

  Consequently, it was decided to neglect the effect of the influent composition in the parameters and on the other hand, to introduce direct formulas that relate pH with the kinetic and stoichiometry values affected. Thus, **the sensitivity analysis allowed to simplify considerably the complex interactions between the parameters** existing in the Metabolic model (Oehmen *et al.*, 2010)**,** without compromising the reliability of the proposed model.

- Due to the inclusion multistep nitrification/denitrification processes, the amounts of intermediates like nitrite and nitrous oxide can be quantified. As a result**, the proposed model may be used to optimize EBPR systems coupled with N removal in order to reduce the accumulation and release of undesirable denitrification intermediates** that present toxicity or contribute to the greenhouse gas effect.

- The comparison between the processes related with PAOs in the ASM2d+TUD model (Meijer, 2004) and the Metabolic model (Oehmen *et al.*, 2010) showed that the stoichiometric values are similar, whereas the maximum rates are considerably different in some cases. Additionally, each model considers different Monod equations and half saturation constant values (K). The latter disagreements in kinetics are common when comparing different models, and it is difficult to predict its impact because it depends on the concentration of the components considered in the K values. So that, **further evaluation of the kinetics for PAOs and GAOs processes is required for the model proposed** in this thesis.

- The software AQUASIM (a simulator for aquatic environments) was used to evaluate the performance of a PAO culture and a mixed PAO-GAO culture by using the proposed model without any calibration of the maximum rate values. The difference between the predicted values in the model and the ones measured in laboratory were 11-13% for acetate, 19-24% for glycogen, 6-7% for PHA, and 17% for P concentrations. The predicted occurrence of PAO-GAO communities for the mixed culture was very similar to the one measured in the laboratory (77-23% and 73-27% respectively). Furthermore, the profiles obtained when pH, temperature and influent composition changes were consistent with the performance reported in the laboratory for PAO-GAO cultures (Lopez-Vazquez *et al.*, 2009). These results suggest that **at least the part used to test the model was reliable**, which corresponds to the competition between PAOs and GAOs for acetate under anaerobic/aerobic conditions. **This approach can be used to test the remaining components of the model before a further calibration and validation of this.**

- **A modification for the existing Johannesburg configuration (called MJHB-IHE in this thesis) was proposed**, which is expected to have lower operational costs compared to existing process configurations due to its simplicity and enhancement of denitrifying PAO activity.

- Despite some of the processes included in this thesis need to be better understood and that fully calibration/validation using an integrated real case of study is required, it is expected that this model can describe in a more accurate and reliable approach EBPR systems, especially those that presents unexpected low P-removal efficiencies. **Common problems in EBPR configuration could be foreseen and reduced by using this model, like the impact of pH and VFA compounds in P removal, the proliferation of competitor microorganisms or the accumulation of denitrification intermediates.**

This can potentially help to evaluate population dynamics and gain a better understanding of their interactions as well to **predict the operating conditions that enrich or restrict certain microbial populations**, which is a key factor to optimize EBPR processes.

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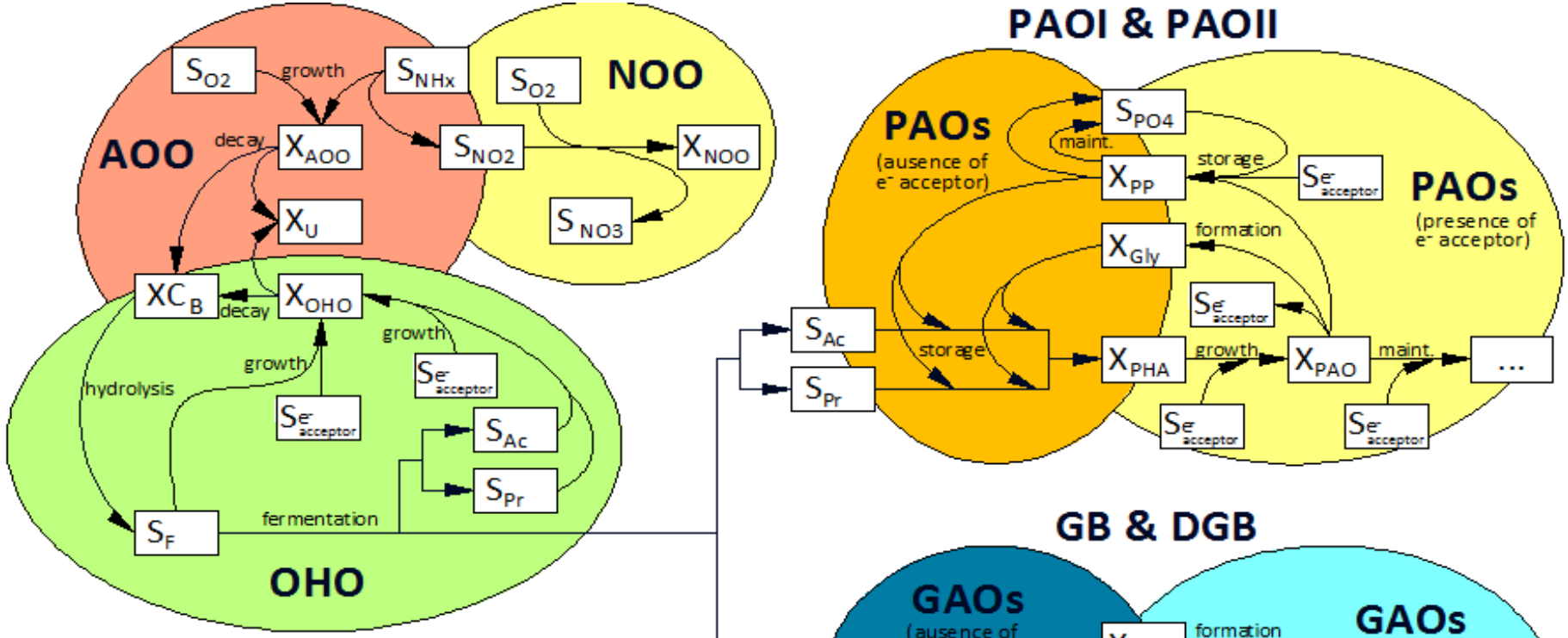
Annex:
Extended Model ASM2d+TUD

A.1 Overview of Extended ASM2d+TUD model



Microorganisms	($S_{e^-_{acceptor}}$) Electron acceptor			
	O_2	NO_3^-	NO_2^-	N_2O
OHOs	X	X	X	X
PAOI	X	X	X	X
PAOII	X		X	X
GB	X			
DGB	X	X	X	X

Figure 22: Interactions in the extended model proposed in this thesis

A.2 State variables

The state variables involved in the integrated model are presented in Table 23:

Table 23: State variables

Soluble parameters	Notation	unit
Fermentable organic matter	S_F	g COD.m^{-3}
Fermentation product (acetate)	S_{Ac}	g COD.m^{-3}
Fermentation product (propionate)	S_{Pr}	g COD.m^{-3}
Soluble undegradable organics	S_U	g COD.m^{-3}
Dissolved oxygen	S_{O2}	- g COD.m^{-3}
Ammonia (NH4+ + NH3)	S_{NHx}	g N.m^{-3}
Nitrate (NO_3)	S_{NO3}	g N.m^{-3}
Nitrite (NO_2)	S_{NO2}	g N.m^{-3}
Nitrous oxide (N2O)	S_{N2O}	g N.m^{-3}
Dissolved nitrogen gas	S_{N2}	g N.m^{-3}
Inorganic soluble phosphorus	S_{PO4}	g P.m^{-3}
Alkalinity ($HCO3^-$)	S_{Alk}	mol HCO_3^-.m^{-3}

Particulate parameters	Notation	unit
Particulate biodegradable organics	XC_B	g COD.m^{-3}
Particulate undegradable organics	X_U	g COD.m^{-3}
Ordinary heterotrophic organisms	X_{OHO}	g COD.m^{-3}
Autotrophic nitrifying organisms (NH4+ to NO3-)	X_{ANO}	g COD.m^{-3}
Phosphorus accumulating organisms, type I	X_{PAOI}	g COD.m^{-3}
Stored poly-β-hydroxyalkanoate in PAOI	$X_{PAOI,PHA}$	g COD.m^{-3}
Stored glycogen in PAOI	$X_{PAOI,Gly}$	g COD.m^{-3}
Stored polyphosphates in PAOI	$X_{PAOI,PP}$	g P.m^{-3}
Phosphorus accumulating organisms, type II	X_{PAOII}	g COD.m^{-3}
Stored poly-β-hydroxyalkanoate in PAOII	$X_{PAOII,PHA}$	g COD.m^{-3}
Stored glycogen in PAOII	$X_{PAOII,Gly}$	g COD.m^{-3}
Stored polyphosphates in PAOII	$X_{PAOII,PP}$	g P.m^{-3}
Glycogen accumulating organisms	X_{GB}	g COD.m^{-3}
Stored poly-β-hydroxyalkanoate in GB	$X_{GB,PHA}$	g COD.m^{-3}
Stored glycogen in GB	$X_{GB,Gly}$	g COD.m^{-3}
Denitrifying Glycogen accumulating organisms	X_{DGB}	g COD.m^{-3}
Stored poly-β-hydroxyalkanoate in DGB	$X_{DGB,PHA}$	g COD.m^{-3}
Stored glycogen in DGB	$X_{DGB,Gly}$	g COD.m^{-3}
Total suspended solids	X_{TSS}	g TSS.m^{-3}

A.3 Conversion factors

Table 24: Stoichiometry values

Abreviation	Compound	Complete formula	Formula base on C-mmol	Value	Units g/mol per C-mmol	Value	Units mgCOD per C-mmol
Ac	Acetic acid	CH3COOH	CH2O	30.03	g/mol for C-mmol	32.00	mgCOD/C-mmol
Pr	Propionic acid	CH3CH2COOH	CH2O2/3	24.69	g/mol for C-mmol	37.33	mgCOD/C-mmol
Gly	Glycogen	C6H10O5	CH10/6O5/6	27.02	g/mol for C-mmol	32.00	mgCOD/C-mmol
Xbio	Biomass		CH2.09O0.54N0.20P0.015	26.02	g/mol for C-mmol	35.88	mgCOD/C-mmol
PHB	poly-β-hydroxybutyrate	CH3CH2CHCO2	CH1.5O0.5	21.52	g/mol for C-mmol	36.00	mgCOD/C-mmol
PHV	poly-β-hydroxyvalerate	CH3CH2CHCH2CO2	CH1.6O0.4	20.02	g/mol for C-mmol	38.40	mgCOD/C-mmol
PH2MV	poly-β-hydroxy-2-methylvalerate	CH3CH2CHCH2CH2CO2	CH5/3O1/3	19.02	g/mol for C-mmol	40.00	mgCOD/C-mmol
PHA	Poly-β-hydroxyalkanoate	PHB + PHV		20.79224729	g/mol for C-mmol	37.17	mgCOD/C-mmol

P	Phosphorus		30.974	gP/gmol
O2	Oxygen		31.998	gCOD/gmol
C	Carbono		12.01	gC/gmol
N	Nitrogen		14.007	gN/gmol
ATP	Adenosine triphosphate	$C_{10}H_{16}N_5O_{13}P_3$	92.922	gP/gmol

A.4 Stoichiometric values

Table 25: Stoichiometry values

Notation	Hydrolisis	unit	Value* T=20°C
$q_{XCB\_SB,hyd}$	Maximum specific hydrolysis rate	g XC_B.g X_{OHO}^{-1}.d^{-1}	3
$K_{XCB,hyd}$	Half saturation parameter for XC_B/X_{OHO}	g XC_B.g X_{OHO}^{-1}	0.1
$n_{qhyd,Ax}$	Correction factor for hydrolysis under anoxic conditions	-	0.8
$n_{qhyd,An}$	Correction factor for hydrolysis under anaerobic conditions	-	0.2
$K_{O2,hyd}$	Half saturation/inhibition parameter for S_{O2}	g S_{O2}.m^{-3}	0.2
$K_{NOx,hyd}$	Half saturation/inhibition parameter for S_{NOx}	g $N.m^{-3}$	0.5
Notation	**Ordinary Heterotrophic Organisms**	**unit**	**Value* T=20°C**
$f_{SU\_XCB,hyd}$	Fraction of inert COD generated in hydrolysis	g S_U.g XC_B^{-1}	0
f_{Ac_VFA}	Fraction of acetate from total volatile fatty acids	g Ac.g VFA^{-1}	0.6
Y_{OHO_Ox}	Yield for X_{OHO} growth under aerobic conditions	g X_{OHO}.g XC_B^{-1}	0.63
Y_{OHO_NO3}	Yield for X_{OHO} growth under anoxic conditions (using NO3)	g X_{OHO}.g XC_B^{-1}	0.67

Y_{OHO_NO2}	Yield for X_{OHO} growth under anoxic conditions (using NO2)	g X_{OHO}.g XC_B^{-1}	0.67
Y_{OHO_N2O}	Yield for X_{OHO} growth under anoxic conditions (using N2O)	g X_{OHO}.g XC_B^{-1}	0.67
$f_{XU\_OHO,lys}$	Fraction of X_U generated in heterotrophic biomass decay	g X_U.g X_{OHO}^{-1}	0.1
$\mu_{OHO,Max}$	Maximum growth rate of X_{OHO}	d^{-1}	6
$n_{\mu OHO,Ax}$	Reduction factor for anoxic growth of X_{OHO}	-	0.8
$q_{SF\_Ac,Max}$	Rate constant for fermentation / Maximum specific fermentation growth rate	g S_F.g X_{OHO}^{-1}.d^{-1}	3
$K_{SF,OHO}$	Half saturation parameter for S_F	g S_F.m^{-3}	4
$K_{Ac,OHO}$	Half saturation parameter for S_{Ac}	g S_{Ac}.m^{-3}	4
m_{OHO}	Endogenous respiration rate of X_{OHO}	d^{-1}	0.4
$K_{SF,fe}$	Half saturation parameter for fermentation of S_F	g S_F.m^{-3}	20
$K_{O2,OHO}$	Half saturation parameter for S_{O2}	g S_{O2}.m^{-3}	0.2
$K_{NOx,OHO}$	Half saturation parameter for S_{NOx}	g S_{NOx}.m^{-3}	0.5
$K_{NHx,OHO}$	Half saturation parameter for S_{NHx}	g S_{NHx}.m^{-3}	0.05
$K_{PO4,OHO}$	Half saturation parameter for S_{PO4}	g S_{PO4}.m^{-3}	0.01
$K_{Alk,OHO}$	Half saturation parameter for S_{Alk}	mol HCO_3^-.m^{-3}	0.1
Notation	**Autotrophic nitrifying organisms**	**unit**	**Value* T=20°C**
Y_{ANO}	Yield of X_{ANO} growth per S_{NO3}	g X_{AUT}.g S_{NOx}^{-1}	0.24
Y_{AOO}	Yield of X_{AOO} growth per S_{NHx}	g X_{AUT}.g S_{NO2}^{-1}	0.2
Y_{NOO}	Yield of X_{NOO} growth per S_{NO3}	g X_{AUT}.g S_{NO3}^{-1}	0.05
$f_{XU\_AOO,lys}$	Fraction of X_U generated in X_{AOO} decay	g X_U.g X_{ANO}^{-1}	0.1
$\mu_{ANO,Max}$	Maximum growth rate of X_{ANO}	d^{-1}	1
$\mu_{AOO,Max}$	Maximum growth rate of X_{AOO}	d^{-1}	1.1
$\mu_{NOO,Max}$	Maximum growth rate of X_{NOO}	d^{-1}	1.8
$K_{O2,ANO}$	Half saturation parameter for S_{O2}	g S_{O2}.m^{-3}	0.5
$K_{NHx,ANO}$	Half saturation parameter for S_{NHx}	g S_{NHx}.m^{-3}	1
$K_{PO4,ANO}$	Half saturation parameter for S_{PO4}	g S_{PO4}.m^{-3}	0.01
Notation	**Composition**	**unit**	**Value* T=20°C**
i_{N_XBio}	N content of biomass (X_{OHO}, X_{PAO}, X_{ANO})	g N.g X_{Bio}^{-1}	0.08
i_{P_XBio}	P content of biomass (X_{OHO}, X_{PAO}, X_{ANO})	g P.g X_{Bio}^{-1}	0.01
$i_{NOx,N2}$	Conversion factor for NO_3 reduction to N_2	g COD.g N^{-1}	2.86
$i_{NO3,NO2}$	Conversion factor for NO_3 reduction to NO_2	g COD.g N^{-1}	1.14
$i_{NO2,N2O}$	Conversion factor for NO_2 reduction to N_2O	g COD.g N^{-1}	1.14
$i_{N2O,N2}$	Conversion factor for N_2O reduction to N_2	g COD.g N^{-1}	0.57
i_{COD_NO3}	Conversion factor for NO_3 in COD	g COD.g N^{-1}	-4.57
i_{COD_NO2}	Conversion factor for NO_2 in COD	g COD.g N^{-1}	-3.43
i_{COD_N2O}	Conversion factor for N_2O in COD	g COD.g N^{-1}	-2.29
i_{COD_N2}	Conversion factor for N_2 in COD	g COD.g N^{-1}	-1.71
i_{Charge_Ac}	Conversion factor for S_{Ac} (CH_3COO^-)in charge	Charge.g COD^{-1}	-0.02
i_{Charge_Pr}	Conversion factor for S_{Pr} ($CH_{3CH2}COO^-$)in charge	Charge.g COD^{-1}	-0.01
i_{Charge_NHx}	Conversion factor for NH_x in charge	Charge.g N^{-1}	0.07
i_{Charge_NO3}	Conversion factor for NO_3 in charge	Charge.g N^{-1}	-0.07
i_{Charge_NO2}	Conversion factor for NO_2 in charge	Charge.g N^{-1}	-0.07

i_{Charge_PO4}	Conversion factor for PO_4 in charge	Charge.g P^{-1}	-0.05
$i_{Charge\_XPAO,PP}$	Conversion factor for $X_{PAO,PP}$ $(K_{0.33}Mg_{0.33}PO_3)_n$ in charge	Charge.g P^{-1}	-0.03

Notation	Description	Units	Value	pH*
Stoichiometry *Accumulibacter* I and II (PAOs)				
$m_{PAO,N2O}$	Nitrous oxide requirements for maintenance per PAO biomass concentration	g N/g COD	1.07	
$m_{PAO,NOx}$	Nitrogen requirements for maintenance per PAO biomass concentration	g N/g COD	0.53	
$m_{PAO,Ox}$	Oxygen requirements for maintenance per PAO biomass concentration	g O2/g COD	0.75	
Notation	**Description**	**Units**	**Value**	**pH***
Stoichiometry *Accumulibacter* I and II (PAOs)				
$Y_{Ac\ PHA,PAO}$	PHA stored per Ac taken up by PAO under anaerobic conditions	g COD/g COD	1.50	
$Y_{Gly\ Ac,PAO}$	Glycogen consumed per Ac taken up by PAO under anaerobic conditions	g COD/g COD	0.50	
$Y_{Gly\ Pr,PAO}$	Glycogen consumed per Pr taken up by PAO under anaerobic conditions	g COD/g COD	0.29	X
$Y_{N2O\ Gly,PAO}$	Nitrous oxide not consumed per PHA degraded due to glycogen formation	g N/g COD	0.33	
$Y_{N2O\ PHA,PAO}$	Nitrous oxide consumed per PHA degraded for PAO biomass synthesis	g N/g COD	0.68	
$Y_{N2O\ PP,PAO}$	Nitrous oxide consumed per PHA degraded due to PP formation	g N/g P	0.61	
$Y_{NOx\ Gly,PAO}$	Nitrate/ Nitrite not consumed per PHA degraded due to glycogen formation	g N/g COD	0.17	
$Y_{NOx\_PHA,PAO}$	Nitrate/Nitrite consumed per PHA degraded for PAO biomass synthesis	g N/g COD	0.34	
$Y_{NOx\ PP,PAO}$	Nitrate/Nitrite consumed per PHA degraded due to PP formation	g N/g P	0.31	
$Y_{O2\ Gly,PAO}$	Oxygen not consumed per PHA degraded due to glycogen formation	g O2/g COD	0.11	
$Y_{O2\ PHA,PAO}$	Oxygen consumed per PHA degraded for PAO biomass synthesis	g O2/g COD	0.25	
$Y_{O2\ PP,PAO}$	Oxygen consumed per PHA degraded due to PP formation	g O2/g P	0.24	
$Y_{PHA\ X,PAO,Ax}$	Maximum PAO biomass production per PHA degraded under anoxic conditions	g COD/g COD	0.61	
$Y_{PHA\ X,PAO,Ox}$	Maximum PAO biomass production per PHA degraded under aerobic conditions	g COD/g COD	0.75	
$Y_{PO4\ Ac,PAO}$	Poly-P consumed per Ac taken up by PAO under anaerobic conditions	g P/g COD	0.39	X
$Y_{PO4\_Pr,PAO}$	Poly-P consumed per Pr taken up by PAO under anaerobic conditions	g P/g COD	0.25	X
$Y_{Pr\ PHA,PAO}$	PHA stored per Pr taken up by PAO under anaerobic conditions	g COD/g COD	1.29	X
$Y_{X\ Gly,PAO,Ax}$	PAO biomass not produced due to Gly formation under anoxic conditions	g COD/g COD	0.81	
$Y_{X\ Gly,PAO,Ox}$	PAO biomass not produced due to Gly formation under aerobic conditions	g COD/g COD	0.89	
$Y_{X\ PP,PAO,Ax}$	PAO biomass not produced due to PP formation under anoxic conditions	g COD/g P	0.35	
$Y_{X\ PP\ PAO\ Ox}$	PAO biomass not produced due to PP formation under aerobic conditions	g COD/g P	0.24	
Stoichiometry *Competibacter* and Denitrifying *Competibacter* (GAOs)				
$Y_{Ac\ PHA,GAO}$	PHA stored per Ac taken up by GB or DGB under anaerobic conditions	g COD/g COD	2.17	X
$Y_{Gly\ Ac,GAO}$	Glycogen consumed per Ac taken up by BG or DGB under anaerobic conditions	g COD/g COD	1.12	X
$Y_{Gly\ Pr,GAO}$	Glycogen consumed per Pr taken up by GB under anaerobic conditions	g COD/g COD	0.57	
$Y_{N2O\ Gly,DGB}$	Nitrous oxide not consumed per PHA degraded due to glycogen formation	g N/g COD	0.34	

$Y_{N2O\_PHA,DGB}$	Nitrous oxide consumed per PHA degraded for DGB biomass synthesis	g N/g COD	0.67	
$Y_{NOx\_Gly,DGB}$	Nitrate/ Nitrite not consumed per PHA degraded due to glycogen formation	g N/g COD	0.17	
$Y_{NOx\_PHA,DGB}$	Nitrate/Nitrite consumed per PHA degraded for DGB biomass synthesis	g N/g COD	0.34	
$Y_{O2\_Gly,GAO}$	Oxygen not consumed per PHA degraded due to glycogen formation	g O2/g COD	0.11	
$Y_{O2\_PHA,GAO}$	Oxygen consumed per PHA degraded for GB / DGB biomass synthesis	g O2/g COD	0.24	
$Y_{PHA\_X,DGB,Ax}$	Maximum DGB biomass production per PHA degraded under anoxic conditions	g COD/g COD	0.62	
$Y_{PHA\_X,GAO,Ox}$	Maximum GB / DGB biomass production per PHA degraded under aerobic conditions	g COD/g COD	0.76	
$Y_{Pr\_PHA,GAO}$	PHA stored per Pr taken up by GB under anaerobic conditions	g COD/g COD	1.51	
$Y_{X\_Gly,DGB,Ax}$	DGB biomass not produced due to Gly formation under anoxic conditions	g COD/g COD	0.81	
$Y_{X\_Gly,GAO,Ox}$	GB biomass not produced due to Gly formation under aerobic conditions	g COD/g COD	0.89	

*pH dependent variables, see Table 26.

Table 26: Yields and rates of PAOs and GAOs that depends on pH.

Notation	Description	Units	pH*						Max. variation	Formula included in the model
			6	6.5	7	7.5	8	8.5		
Stoichiometry of PAOI and PAO II (PAOs)										
$Y_{Gly\_Pr,PAO}$	Glycogen consumed per Pr taken up by PAO under anaerobic conditions	g COD/g COD	0.29	0.29	**0.29**	0.20	0.11	0.03	91%	if pH>7 then (1.483-0.171*pH) else (0.283) endif
$Y_{PO4\_Ac,PAO}$	Poly-P consumed per Ac taken up by PAO under anaerobic conditions	g P/g COD	0.20	0.30	**0.39**	0.48	0.57	0.66	71%	-0.9+0.184*pH
$Y_{PO4\_Pr,PAO}$	Poly-P consumed per Pr taken up by PAO under anaerobic conditions	g P/g COD	0.25	0.25	**0.25**	0.25	0.33	0.41	67%	if pH>7.5 then (-0.996+ 0.166*pH) else (0.249) endif
$Y_{Pr\_PHA\_PAO}$ ***(note 1)***	PHA stored per Pr taken up by PAO under anaerobic conditions	g COD/g COD	1.29	1.29	**1.29**	1.20	1.11	1.03	20%	if pH>7 then (2.495-0.173*pH) else (1.285) endif
Stoichiometry of GB and DGB (GAOs)										
$Y_{Ac\_PHA,GAO}$	PHA stored per Ac taken up by GB or DGB under anaerobic conditions	g COD/g COD	2.07	2.12	**2.17**	2.22	2.27	2.31	7%	1.469+0.1*pH
$Y_{Gly\_Ac,GAO}$	Glycogen consumed per Ac taken up by GAOs under anaerobic conditions	g COD/g COD	1.00	1.06	**1.12**	1.18	1.23	1.29	15%	0.32+0.114*pH
Kinetics of GB and DGB (GAOs)										
$q_{DGB,Ac\_PHA,20}$	Maximum acetate uptake rate for DGB at 20°C	g COD/g COD/d	2.37	2.31	**2.14**	1.74	1.09	0.50	77%	-0.3265*pH^2 + 3.9677*pH - 9.6739
$q_{GB,Ac\_PHA,20}$	Maximum acetate uptake rate for GB at 20°C	g COD/g COD/d	4.74	4.62	**4.28**	3.48	2.18	1.00	77%	-0.653* pH^2 + 7.9351*pH - 19.347
$q_{DGB,Pr\_PHA,20}$	Maximum propionate uptake rate for DGB at 20°C	g COD/g COD/d	0.14	0.13	**0.12**	0.10	0.06	0.03	77%	-0.019*pH^2+ 0.2313*pH - 0.564
$q_{GB,Pr\_PHA,20}$	Maximum propionate uptake rate for GB at 20°C	g COD/g COD/d	0.28	0.27	**0.25**	0.20	0.13	0.06	77%	-0.0381*pH^2 + 0.4626*pH - 1.1279

Note 1. The calculation of this parameter depending on pH was corrected in this model. See Chpater 4.2.2. of this thesis

Note 2. The alkalinity was calculated in the model considering the charges contributed by the different species produced or consumed during each process.

A.5 Kinetic values

Table 27: Kinetic values

Notation	Description	Units		pH
Kinetics *Accumulibacter* I and II (PAOs)				
$m_{PAO\_PHA,Ax,20}$	Nitrogen necessary for aerobic maintenance purposes of PAO at 20oC	g N/g COD/d	0.09	
$m_{PAO\_PHA,Ox,20}$	Oxygen necessary for aerobic maintenance purposes of PAO at 20oC	g O2/g COD/d	0.09	
$m_{PAO,PP,An,20}$	ATP necessary for anaerobic maintenance purposes of PAOs at 20oC	g P/g COD/d	0.05	
$q_{PAO,Gly\_Ac,Ax,20}$	Glycogen_Ac formation by PAO under anoxic conditions	g COD/g COD/d	0.43	
$q_{PAO,Gly\_Ac,Ox,20}$	Glycogen_Ac formation by PAO under aerobic conditions	g COD/g COD/d	0.32	
$q_{PAO,Gly\_Pr,Ax,20}$	Glycogen_Pr formation by PAO under anoxic conditions	g COD/g COD/d	0.05	
$q_{PAO,Gly\_Pr,Ox,20}$	Glycogen_Pr formation by PAO under aerobic conditions	g COD/g COD/d	0.16	
$q_{PAO,PHA\_Ac,Ax,20}$	PHA_Ac degradation rate by PAO under anoxic conditions	g COD/g COD/d	7.46	
$q_{PAO,PHA\_Ac,Ox,20}$	PHA_Ac degradation rate by PAO under aerobic conditions	g COD/g COD/d	19.89	
$q_{PAO,PHA\_Pr,Ax,20}$	PHA_Pr degradation rate by PAO under anoxic conditions	g COD/g COD/d	1.24	
$q_{PAO,PHA\_Pr,Ox,20}$	PHA_Pr degradation rate by PAO under aerobic conditions	g COD/g COD/d	8.20	
$q_{PAO,PO4\_PP\_Ac,Ax,20}$	Anoxic P-uptake rate by PAO, depending on acetate in the influent (Std. cond)	g P/g COD/d	0.10	
$q_{PAO,PO4\_PP\_Ac,Ox,20}$	Aerobic P-uptake rate by PAO, depending on acetate in the influent (Std. cond)	g P/g COD/d	0.41	
$q_{PAO,PO4\_PP\_Pr,Ax,20}$	Anoxic P-uptake rate by PAO, depending on propionate in the influent (Std. cond)	g P/g COD/d	0.02	
$q_{PAO,PO4\_PP\_Pr,Ox,20}$	Aerobic P-uptake rate by PAO, depending on propionate in the influent (Std. cond)	g P/g COD/d	0.04	
$q_{PAOI,Ac\_PHA,20}$	Maximum acetate uptake rate for PAOI at 20oC	g COD/g COD/d	2.14	
$q_{PAOI,Pr\_PHA,20}$	Maximum propionate uptake rate for PAOI at 20oC	g COD/g COD/d	2.50	
$q_{PAOII,Ac\_PHA,20}$	Maximum acetate uptake rate for PAOII at 20oC	g COD/g COD/d	4.28	
$q_{PAOII,Pr\_PHA,20}$	Maximum propionate uptake rate for PAOII at 20oC	g COD/g COD/d	4.99	
Kinetics *Competibacter* and Denitrifying *Competibacter* (GAOs)				
$m_{DGB\_PHA,Ax,20}$	Nitrogen necessary for aerobic maintenance purposes of DGB at 20oC	g N/g COD/d	0.09	
$m_{GAO\_PHA,Ox,20}$	Oxygen necessary for aerobic maintenance purposes of GB at 20oC	g O2/g COD/d	0.09	
$m_{GAO,Gly,An,20}$	Glycogen necessary for anaerobic maintenance purposes of GB and DGB at 20oC	g COD/g COD/d	0.10	
$q_{DGB,Ac\_PHA,20}$	Maximum acetate uptake rate for DGB at 20oC	g COD/g COD/d	2.14	X
$q_{DGB\_PHA,Ax,20}$	PHA degradation rate by DGB under anoxic conditions	g COD/g COD/d	10.53	
$q_{DGB,Pr\_PHA,20}$	Maximum propionate uptake rate for DGB at 20oC	g COD/g COD/d	0.12	X
$q_{DGB,Gly,Ax,20}$	Glycogen formation by GAO under anoxic conditions	g COD/g COD/d	0.78	
$q_{GAO,Gly,Ox,20}$	Glycogen formation by GAO under aerobic conditions	g COD/g COD/d	6.42	
$q_{GAO,PHA,Ox,20}$	PHA degradation rate by GB / DGB under aerobic conditions	g COD/g COD/d	27.57	
$q_{GB,Ac\_PHA,20}$	Maximum acetate uptake rate for GB at 20oC	g COD/g COD/d	4.28	X
$q_{GB,Pr\_PHA,20}$	Maximum propionate uptake rate for GB at 20oC	g COD/g COD/d	0.25	X

Table 28: Fractions for kinetic values

Notation	Description	Units	Value	pH
$f_{PAO\_Gly,Max}$	Maximum glycogen content per PAO biomass concentration	g COD/g COD	0.24	
$f_{PAO\_PP,Max}$	Maximum Poly-P content per PAO biomass concentration	g P/g COD	0.26	
$f_{GAO\_Gly,Max}$	Maximum glycogen content per GB/DGB biomass concentration	g COD/g COD	0.31	
$f_{PHA,Max}$	Maximum PHA content per PAO and GAO biomass concentration	g COD/g COD	1.04	

A.6 Stoichiometry and composition matrix

Table 29: Stoichiometry and composition matrix for OHOs and ANOs (AOO and NOO)

Hydrolysis processes		S_F	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	S_{Alk}	X_{OHO}	X_{AOO}	X_{NOO}	XC_B	X_U	S_U
1	Aerobic hydrolysis	$1-f_{SU\_XCB,hyd}$								$i_{N\_XCB}-i_{N\_SU}*f_{SU\_XCB,hyd}-i_{N\_SF}*(1-f_{SU\_XCB,hyd})$	$i_{P\_XCB}-i_{P\_SU}*f_{SU\_XCB,hyd}-i_{P\_SF}*(1-f_{SU\_XCB,hyd})$	$Y_{Alk\_hyd,Ox}$				-1		$f_{SU\_XCB,hyd}$
2	Anoxic hydrolysis	$1-f_{SU\_XCB,hyd}$								$i_{N\_XCB}-i_{N\_SU}*f_{SU\_XCB,hyd}-i_{N\_SF}*(1-f_{SU\_XCB,hyd})$	$i_{P\_XCB}-i_{P\_SU}*f_{SU\_XCB,hyd}-i_{P\_SF}*(1-f_{SU\_XCB,hyd})$	$Y_{Alk\_hyd,Ax}$				-1		$f_{SU\_XCB,hyd}$
3	Anaerobic hydrolysis	$1-f_{SU\_XCB,hyd}$								$i_{N\_XCB}-i_{N\_SU}*f_{SU\_XCB,hyd}-i_{N\_SF}*(1-f_{SU\_XCB,hyd})$	$i_{P\_XCB}-i_{P\_SU}*f_{SU\_XCB,hyd}-i_{P\_SF}*(1-f_{SU\_XCB,hyd})$	$Y_{Alk\_hyd,An}$				-1		$f_{SU\_XCB,hyd}$
Ordinary Heterotrophic Organisms (OHO)		S_F	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	S_{Alk}	X_{OHO}	X_{AOO}	X_{NOO}	XC_B	X_U	S_U
4	Aerobic growth of X_{OHO} on S_F	$-1/Y_{OHO,Ox}$			$-(1-Y_{OHO,Ox})/Y_{OHO,Ox}$					$i_{N\_SF}/Y_{OHO,Ox}-i_{N\_XBio}$	$i_{P\_SF}/Y_{OHO,Ox}-i_{P\_XBio}$	$Y_{Alk\_F,OHO,Ox}$	1					
5	Aerobic growth of X_{OHO} on S_{VFA}		$-f_{Ac\_VFA}/Y_{OHO,Ox}$	$-(1-f_{Ac\_VFA})/Y_{OHO,Ox}$	$-(1-Y_{OHO,Ox})/Y_{OHO,Ox}$					$-i_{N\_XBio}$	$-i_{P\_XBio}$	$-Y_{Alk\_VFA,OHO,Ox}$	1					
6	Anoxic growth of X_{OHO} on S_F (using NO_3)	$-1/Y_{OHO,NO3}$				$-(1-Y_{OHO,NO3})/(i_{NO3,NO2}*Y_{OHO,NO3})$	$(1-Y_{OHO,NO3})/(i_{NO3,NO2}*Y_{OHO})$			$i_{N\_SF}/Y_{OHO,NO3}-i_{N\_XBio}$	$i_{P\_SF}/Y_{OHO,NO3}-i_{P\_XBio}$	$-Y_{Alk\_F,OHO,NO3}$	1					
7	Anoxic growth of X_{OHO} on S_F (using NO_2)	$-1/Y_{OHO,NO2}$					$-(1-Y_{OHO,NO2})/(i_{NO2,N2O}*Y_{OHO,NO2})$	$(1-Y_{OHO,NO2})/(i_{NO2,N2O}*Y_{OHO,NO2})$		$i_{N\_SF}/Y_{OHO,NO2}-i_{N\_XBio}$	$i_{P\_SF}/Y_{OHO,NO2}-i_{P\_XBio}$	$Y_{Alk\_F,OHO,NO2}$	1					
8	Anoxic growth of X_{OHO} on S_F (using N_2O)	$-1/Y_{OHO,N2O}$						$-(1-Y_{OHO,N2O})/(i_{N2O,N2}*Y_{OHO,N2O})$	$(1-Y_{OHO,N2O})/(i_{N2O,N2}*Y_{OHO,N2O})$	$i_{N\_SF}/Y_{OHO,N2O}-i_{N\_XBio}$	$i_{P\_SF}/Y_{OHO,N2O}-i_{P\_XBio}$	$-Y_{Alk\_F,OHO,N2O}$	1					
9	Anoxic growth of X_{OHO} on S_{VFA} (using NO_3)		$-f_{Ac\_VFA}/Y_{OHO,NO3}$	$-(1-f_{Ac\_VFA})/Y_{OHO,NO3}$		$-(1-Y_{OHO,NO3})/(i_{NO3,NO2}*Y_{OHO,NO3})$	$(1-Y_{OHO,NO3})/(i_{NO3,NO2}*Y_{OHO,NO3})$			$-i_{N\_XBio}$	$-i_{P\_XBio}$	$Y_{Alk\_VFA,OHO,NO3}$	1					
10	Anoxic growth of X_{OHO} on S_{VFA} (using NO_2)		$-f_{Ac\_VFA}/Y_{OHO,NO2}$	$-(1-f_{Ac\_VFA})/Y_{OHO,NO2}$			$-(1-Y_{OHO,NO2})/(i_{NO2,N2O}*Y_{OHO,NO2})$	$(1-Y_{OHO,NO2})/(i_{NO2,N2O}*Y_{OHO,NO2})$		$-i_{N\_XBio}$	$-i_{P\_XBio}$	$Y_{Alk\_VFA,OHO,NO2}$	1					
11	Anoxic growth of X_{OHO} on S_{VFA} (using N_2O)		$-f_{Ac\_VFA}/Y_{OHO,N2O}$	$-(1-f_{Ac\_VFA})/Y_{OHO,N2O}$				$-(1-Y_{OHO,N2O})/(i_{N2O,N2}*Y_{OHO,N2O})$	$(1-Y_{OHO,N2O})/(i_{N2O,N2}*Y_{OHO,N2O})$	$-i_{N\_XBio}$	$-i_{P\_XBio}$	$Y_{Alk\_VFA,OHO,N2O}$	1					
12	Fermentation	-1	f_{Ac_VFA}	$1-f_{Ac\_VFA}$						i_{N_SF}	i_{P_SF}	$-Y_{AlkF\_VFA,OHO}$						
13	Lysis									$i_{N\_XBio}-i_{N\_XU}*f_{XU\_OHO,lys}-i_{N\_XCB}*(1-f_{XU\_OHO,lys})$	$i_{P\_XBio}-i_{P\_XU}*f_{XU\_OHO,lys}-i_{P\_XCB}*(1-f_{XU\_OHO,lys})$	$Y_{Alk\_lys,OHO}$	-1			$1-f_{XU\_OHO,lys}$	$f_{XU\_OHO,lys}$	
Nitrification (AOO and NOO)		S_F	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	S_{Alk}	X_{OHO}	X_{AOO}	X_{NOO}	XC_B	X_U	S_U
14	Aerobic growth of X_{AOO} on NH_x				$-(-i_{COD\_NO2}-Y_{AOO})/Y_{AOO}$		$1/Y_{AOO}$			$-i_{N\_XBio}-1/Y_{AOO}$	$-i_{P\_XBio}$	$-Y_{Alk\_NO2,AOO}$		1				
15	Aerobic growth of X_{NOO} on NO_2				$-(-(i_{COD\_NO3}-i_{COD\_NO2})-Y_{NOO})/Y_{NOO}$	$1/Y_{NOO}$	$-1/Y_{NOO}$			$-i_{N\_XBio}$	$-i_{P\_XBio}$	$-Y_{Alk\_NO3,NOO}$			1			
16	Lysis X_{AOO}									$i_{N\_XBio}-i_{N\_XU}*f_{XU\_AOO,lys}-i_{N\_XCB}*(1-f_{XU\_NOO,lys})$	$i_{P\_XBio}-i_{P\_XU}*f_{XU\_AOO,lys}-i_{P\_XCB}*(1-f_{XU\_NOO,lys})$	$Y_{Alk\_lys,AOO}$		-1		$1-f_{XU\_AOO,lys}$	$f_{XU\_AOO,lys}$	
COMPOSITION MATRIX		S_F	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	S_{Alk}	X_{OHO}	X_{AOO}	X_{NOO}	XC_B	X_U	S_U
COD	gCOD/g	1	1	1	-1	i_{COD_NO3}	i_{COD_NO2}	i_{COD_N2O}	i_{COD_N2}				1	1	1	1	1	1
Nitrogen	gN/g	i_{N_SF}				1	1	1	1	1			i_{N_XBio}	i_{N_XBio}	i_{N_XBio}	i_{N_XCB}	i_{N_XU}	i_{N_SU}
Phosphorus	gP/g	i_{P_SF}									1		i_{P_XBio}	i_{P_XBio}	i_{P_XBio}	i_{P_XCB}	i_{P_XU}	i_{P_SU}
Ionic charge	charge/g		i_{Charge_Ac}	i_{Charge_Pr}		i_{Charge_NO3}	i_{Charge_NO2}			i_{Charge_NHx}	i_{Charge_PO4}	-1						

Table 30: Stoichiometry and composition matrix for PAOs (PAOI and PAOII) *pH dependent variables.*

	#	Accumulibacter type I (PAO I)	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{PAOI}	X_{PAOI_PHA}	X_{PAOI_Gly}	X_{PAOI_PP}	S_{Alk}
Anaerobic processes DPAO	17	Anaerobic acetate uptake	-1								$Y_{PO4\_Ac,PAO}$		$Y_{Ac\_PHA,PAO}$	$-Y_{Gly\_Ac,PAO}$	$-Y_{PO4\_Ac,PAO}$	$Y_{\_Alk\_Ac,PAO}$
	18	Anaerobic propionate uptake		-1							$Y_{PO4\_Pr,PAO}$		$Y_{Pr\_PHA,PAO}$	$-Y_{Gly\_Pr,PAO}$	$-Y_{PO4\_Pr,PAO}$	$Y_{\_Alk\_Pr,PAO}$
	19	Anaerobic maintenance (PP)									1				-1	$-Y_{Alk\_PP,PAO}$
Aerobic processes DPAO	20	Aerobic PHA degradation			$-Y_{O2\_PHA,PAO}$					$-i_{N\_XBio}*Y_{PHA\_X,PAO,Ox}$	$-i_{P\_XBio}*Y_{PHA\_X,PAO,Ox}$	$Y_{PHA\_X,PAO,Ox}$	-1			$-Y_{Alk\_PHA,PAO,Ox}$
	21	Aerobic glycogen production			$Y_{O2\_Gly,PAO}$					$i_{N\_XBio}*Y_{X\_Gly,PAO,Ox}$	$i_{P\_XBio}*Y_{X\_Gly,PAO,Ox}$	$-Y_{X\_Gly,PAO,Ox}$		1		$Y_{Alk\_Gly,PAO,Ox}$
	22	Aerobic Poly-P formation			$-Y_{O2\_PP,PAO}$					$i_{N\_XBio}*Y_{X\_PP,PAO,Ox}$	$i_{P\_XBio}*Y_{X\_PP\_PAO,Ox}-1$	$-Y_{X\_PP,PAO,Ox}$			1	$Y_{Alk\_PP,PAO,Ox}$
	23	Aerobic maintenance			$-m_{PAO,Ox}$					i_{NXBio}	i_{P_XBio}	-1				$Y_{Alk\_m,PAO,Ox}$
NO3 reduction processes DPAO	24	Anoxic PHA degradation (on NO_3^-)				$-Y_{NOx\_PHA,PAO}$	$Y_{NOx\_PHA,PAO}$			$-i_{\_NXBio}*Y_{PHA\_X,PAO,Ax}$	$-i_{P\_XBio}*Y_{PHA\_X,PAO,Ax}$	$Y_{PHA\_X,PAO,Ax}$	-1			$-Y_{Alk\_PHA,PAO,NO3}$
	25	Anoxic glycogen production (on NO_3^-)				$Y_{NOx\_Gly,PAO}$	$-Y_{NOx\_Gly,PAO}$			$i_{\_NXBio}*Y_{X\_Gly,PAO,Ax}$	$i_{P\_XBio}*Y_{X\_Gly,PAO,Ax}$	$-Y_{X\_Gly,PAO,Ax}$		1		$Y_{Alk\_Gly,PAO,NO3}$
	26	Anoxic Poly-P formation (on NO_3^-)				$-Y_{NOx\_PP,PAO}$	$Y_{NOx\_PP,PAO}$			$i_{\_NXBio}*Y_{X\_PP,PAO,Ax}$	$i_{P\_XBio}*Y_{X\_PP,PAO,Ax}-1$	$-Y_{X\_PP,PAO,Ax}$			1	$Y_{Alk\_PP,PAO,NO3}$
	27	Anoxic maintenance (on NO_3^-)				$-m_{PAO,NOx}$	$m_{PAO,NOx}$			i_{N_XBio}	i_{P_XBio}	-1				$Y_{Alk\_m,PAO,NO3}$
NO2 reduction processes DPAO	28	Anoxic PHA degradation (on NO_2^-)					$-Y_{NOx\_PHA,PAO}$	$Y_{NOx\_PHA,PAO}$		$-i_{N\_XBio}*Y_{PHA\_X,PAO,Ax}$	$-i_{P\_XBio}*Y_{PHA\_X,PAO,Ax}$	$Y_{PHA\_X,PAO,Ax}$	-1			$Y_{Alk\_PHA,PAO,NO2}$
	29	Anoxic glycogen production (on NO_2^-)					$Y_{NOx\_Gly,PAO}$	$-Y_{NOx\_Gly,PAO}$		$i_{N\_XBio}*Y_{X\_Gly,PAO,Ax}$	$i_{P\_XBio}*Y_{X\_Gly,PAO,Ax}$	$-Y_{X\_Gly,PAO,Ax}$		1		$-Y_{Alk\_Gly,PAO,NO2}$
	30	Anoxic Poly-P formation (on NO_2^-)					$-Y_{NOx\_PP,PAO}$	$Y_{NOx\_PP,PAO}$		$i_{N\_XBio}*Y_{X\_PP,PAO,Ax}$	$i_{P\_XBio}*Y_{X\_PP,PAO,Ax}-1$	$-Y_{X\_PP,PAO,Ax}$			1	$Y_{Alk\_PP,PAO,NO2}$
	31	Anoxic maintenance (on NO_2^-)					$-m_{PAO,NOx}$	$m_{PAO,NOx}$		i_{N_XBio}	i_{P_XBio}	-1				$Y_{Alk\_m,PAO,NO2}$
N2O reduction processes DPAO	32	Anoxic PHA degradation (on N_2O)						$-Y_{N2O\_PHA,PAO}$	$Y_{N2O\_PHA,PAO}$	$-i_{N\_XBio}*Y_{PHA\_X,PAO,Ax}$	$-i_{P\_XBio}*Y_{PHA\_X,PAO,Ax}$	$Y_{PHA\_X,PAO,Ax}$	-1			$-Y_{Alk\_PHA,PAO,N2O}$
	33	Anoxic glycogen production (on N_2O)						$Y_{N2O\_Gly,PAO}$	$-Y_{N2O\_Gly,PAO}$	$i_{N\_XBio}*Y_{X\_Gly,PAO,Ax}$	$i_{P\_XBio}*Y_{X\_Gly,PAO,Ax}$	$-Y_{X\_Gly,PAO,Ax}$		1		$Y_{Alk\_Gly,PAO,N2O}$
	34	Anoxic Poly-P formation (on N_2O)						$-Y_{N2O\_PP,PAO}$	$Y_{N2O\_PP,PAO}$	$i_{N\_XBio}*Y_{X\_PP,PAO,Ax}$	$i_{P\_XBio}*Y_{X\_PP,PAO,Ax}-1$	$-Y_{X\_PP,PAO,Ax}$			1	$Y_{Alk\_PP,PAO,N2O}$
	35	Anoxic maintenance (on N_2O)						$-m_{PAO,N2O}$	$m_{PAO,N2O}$	i_{N_XBio}	i_{P_XBio}	-1				$Y_{Alk\_m,PAO,N2O}$
	#	Accumulibacter type II (PAO II)	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{PAOII}	X_{PAOII_PHA}	X_{PAOII_Gly}	X_{PAOII_PP}	S_{Alk}
Anaerobic processes PAO	36	Anaerobic acetate uptake	-1								$Y_{PO4\_Ac,PAO}$		$Y_{Ac\_PHA,PAO}$	$-Y_{Gly\_Ac,PAO}$	$-Y_{PO4\_Ac,PAO}$	$Y_{Alk\_Ac,PAO}$
	37	Anaerobic propionate uptake		-1							$Y_{PO4\_Pr,PAO}$		$Y_{Pr\_PHA,PAO}$	$-Y_{Gly\_Pr,PAO}$	$-Y_{PO4\_Pr,PAO}$	$Y_{Alk\_Pr,PAO}$
	38	Anaerobic maintenance (PP)									1				-1	$-Y_{Alk\_PP,PAO}$
Aerobic processes PAO	39	Aerobic PHA degradation			$-Y_{O2\_PHA,PAO}$					$-i_{N\_XBio}*Y_{PHA\_X,PAO,Ox}$	$-i_{P\_XBio}*Y_{PHA\_X,PAO,Ox}$	$Y_{PHA\_X,PAO,Ox}$	-1			$-Y_{Alk\_PHA,PAO,Ox}$
	40	Aerobic glycogen production			$Y_{O2\_Gly,PAO}$					$i_{N\_XBio}*Y_{X\_Gly,PAO,Ox}$	$i_{P\_XBio}*Y_{X\_Gly,PAO,Ox}$	$-Y_{X\_Gly,PAO,Ox}$		1		$Y_{Alk\_Gly,PAO,Ox}$
	41	Aerobic Poly-P formation			$-Y_{O2\_PP,PAO}$					$i_{N\_XBio}*Y_{X\_PP,PAO,Ox}$	$i_{P\_XBio}*Y_{X\_PP,PAO,Ox}-1$	$-Y_{X\_PP,PAO,Ox}$			1	$Y_{Alk\_PP,PAO,Ox}$
	42	Aerobic maintenance			$-m_{PAO,Ox}$					i_{N_XBio}	i_{P_XBio}	-1				$Y_{Alk\_m,PAO,Ox}$
NO2 reduction processes PAO	43	Anoxic PHA degradation (on NO_2^-)					$-Y_{NOx\_PHA,PAO}$	$Y_{NOx\_PHA,PAO}$		$-i_{N\_XBio}*Y_{PHA\_X,PAO,Ax}$	$-i_{P\_XBio}*Y_{PHA\_X,PAO,Ax}$	$Y_{PHA\_X,PAO,Ax}$	-1			$Y_{Alk\_PHA,PAO,NO2}$
	44	Anoxic glycogen production (on NO_2^-)					$Y_{NOx\_Gly,PAO}$	$-Y_{NOx\_Gly,PAO}$		$i_{N\_XBio}*Y_{X\_Gly,PAO,Ax}$	$i_{P\_XBio}*Y_{X\_Gly,PAO,Ax}$	$-Y_{X\_Gly,PAO,Ax}$		1		$-Y_{Alk\_Gly,PAO,NO2}$
	45	Anoxic Poly-P formation (on NO_2^-)					$-Y_{NOx\_PP,PAO}$	$Y_{NOx\_PP,PAO}$		$i_{N\_XBio}*Y_{X\_PP,PAO,Ax}$	$i_{P\_XBio}*Y_{X\_PP,PAO,Ax}-1$	$-Y_{X\_PP,PAO,Ax}$			1	$Y_{Alk\_PP,PAO,NO2}$
	46	Anoxic maintenance (on NO_2^-)					$-m_{PAO,NOx}$	$m_{PAO,NOx}$		i_{N_XBio}	i_{P_XBio}	-1				$Y_{Alk\_m,PAO,NO2}$
N2O reduction processes PAO	47	Anoxic PHA degradation (on N_2O)						$-Y_{N2O\_PHA,PAO}$	$Y_{N2O\_PHA,PAO}$	$-i_{N\_XBio}*Y_{PHA\_X,PAO,Ax}$	$-i_{P\_XBio}*Y_{PHA\_X,PAO,Ax}$	$Y_{PHA\_X,PAO,Ax}$	-1			$-Y_{Alk\_PHA,PAO,N2O}$
	48	Anoxic glycogen production (on N_2O)						$Y_{N2O\_Gly,PAO}$	$-Y_{N2O\_Gly,PAO}$	$i_{N\_XBio}*Y_{X\_Gly,PAO,Ax}$	$i_{P\_XBio}*Y_{X\_Gly,PAO,Ax}$	$-Y_{X\_Gly,PAO,Ax}$		1		$Y_{Alk\_Gly,PAO,N2O}$
	49	Anoxic Poly-P formation (on N_2O)						$-Y_{N2O\_PP,PAO}$	$Y_{N2O\_PP,PAO}$	$i_{N\_XBio}*Y_{X\_PP,PAO,Ax}$	$i_{P\_XBio}*Y_{X\_PP,PAO,Ax}-1$	$-Y_{X\_PP,PAO,Ax}$			1	$Y_{Alk\_PP,PAO,N2O}$
	50	Anoxic maintenance (on N_2O)						$-m_{PAO,N2O}$	$m_{\_PAO,N2O}$	$i_{\_N\_XBio}$	$i_{\_P\_XBio}$	-1				$Y_{Alk\_m,PAO,N2O}$
		COMPOSITION MATRIX	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{PAOII}	X_{PAOII_PHA}	X_{PAOII_Gly}	X_{PAOII_PP}	S_{Alk}
COD		gCOD/g	1	1	-1	i_{COD_NO3}	i_{COD_NO2}	i_{COD_N2O}	i_{COD_N2}			1	1	1		
Nitrogen		gN/g				1	1	1	1	1		i_{N_XBio}				
Phosphorus		gP/g									1	i_{P_XBio}			1	
Ionic charge		charge/g	i_{Charge_Ac}	i_{Charge_Pr}		i_{Charge_NO3}	i_{Charge_NO2}			i_{Charge_NHx}	i_{Charge_PO4}				$i_{Charge\_XPAO,PP}$	-1

Table 31: Stoichiometry and composition matrix for GAOs (GB and DGB)

	#	Competibacter (GB)	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{GB}	X_{GB_PHA}	X_{GB_Gly}	S_{Alk}
Anaerobic processes GB	51	Anaerobic acetate uptake	-1										$Y_{Ac\_PHA,GAO}$	$-Y_{Gly\_Ac,GAO}$	$Y_{Alk\_Ac\_GAO}$
	52	Anaerobic propionate uptake		-1									$Y_{Pr\_PHA,GAO}$	$-Y_{Gly\_Pr,GAO}$	$Y_{Alk\_Pr\_GAO}$
	53	Anaerobic maintenance											1	-1	
Aerobic processes GB	54	Aerobic PHA degradation			$-Y_{O2\_PHA,GAO}$					$-i_{N\_XBio}*Y_{PHA\_X,GAO,Ox}$	$-i_{P\_XBio}*Y_{PHA\_X,GAO,Ox}$	$Y_{PHA\_X,GAO,Ox}$	-1		$-Y_{Alk\_PHA\_GAO\_Ox}$
	55	Aerobic glycogen production			$Y_{O2\_Gly,GAO}$					$i_{N\_XBio}*Y_{X\_Gly,GAO,Ox}$	$i_{P\_XBio}*Y_{X\_Gly,GAO,Ox}$	$-Y_{X\_Gly,GAO,Ox}$		1	$Y_{Alk\_Gly\_GAO\_Ox}$
	56	Aerobic maintenance			$-m_{GAO,Ox}$					i_{N_XBio}	i_{P_XBio}	-1			$Y_{Alk\_m\_GAO\_Ox}$
	#	Denitrifying Competibacter (DGB)	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{DGB}	X_{DGB_PHA}	X_{DGB_Gly}	S_{Alk}
Anaerobic processes DGB	57	Anaerobic acetate uptake	-1										$Y_{Ac\_PHA,GAO}$	$-Y_{Gly\_Ac,GAO}$	$Y_{Alk\_Ac\_GAO}$
	58	Anaerobic propionate uptake		-1									$Y_{Pr\_PHA,GAO}$	$-Y_{Gly\_Pr,GAO}$	$Y_{Alk\_Pr\_GAO}$
	59	Anaerobic maintenance											1	-1	
Aerobic processes DGB	60	Aerobic PHA degradation			$-Y_{O2\_PHA,GAO}$					$-i_{N\_XBio}*Y_{PHA\_X,GAO,Ox}$	$-i_{P\_XBio}*Y_{PHA\_X,GAO,Ox}$	$Y_{PHA\_X,GAO,Ox}$	-1		$-Y_{Alk\_PHA\_GAO\_Ox}$
	61	Aerobic glycogen production			$Y_{O2\_Gly,GAO}$					$i_{N\_XBio}*Y_{X\_Gly,GAO,Ox}$	$i_{P\_XBio}*Y_{X\_Gly,GAO,Ox}$	$-Y_{X\_Gly,GAO,Ox}$		1	$Y_{Alk\_Gly\_GAO\_Ox}$
	62	Aerobic maintenance			$-m_{GAO,Ox}$					i_{N_XBio}	i_{P_XBio}	-1			$Y_{Alk\_m\_GAO\_Ox}$
NO3 red. processes DGB	63	Anoxic PHA degradation (on NO_3^-)				$-Y_{NOx\_PHA,DGB}$	$Y_{NOx\_PHA,DGB}$			$-i_{N\_XBio}*Y_{PHA\_X,DGB,Ax}$	$-i_{P\_XBio}*Y_{PHA\_X,DGB,Ax}$	$Y_{PHA\_X,DGB,Ax}$	-1		$-Y_{Alk\_PHA\_DGB\_NO3}$
	64	Anoxic glycogen production (on NO_3^-)				$Y_{NOx\_Gly,DGB}$	$-Y_{NOx\_Gly,DGB}$			$i_{N\_XBio}*Y_{X\_Gly,DGB,Ax}$	$i_{P\_XBio}*Y_{X\_Gly,DGB,Ax}$	$-Y_{X\_Gly,DGB,Ax}$		1	$Y_{Alk\_Gly\_DGB\_NO3}$
	65	Anoxic maintenance (on NO_3^-)				$-m_{DGB,NOx}$	$m_{DGB,NOx}$			i_{N_XBio}	i_{P_XBio}	-1			$Y_{Alk\_m\_DGB\_NO3}$
NO2 red. processes DGB	66	Anoxic PHA degradation (on NO_2^-)					$-Y_{NOx\_PHA,DGB}$	$Y_{NOx\_PHA,DGB}$		$-i_{N\_XBio}*Y_{PHA\_X,DGB,Ax}$	$-i_{P\_XBio}*Y_{PHA\_X,DGB,Ax}$	$Y_{PHA\_X,DGB,Ax}$	-1		$Y_{Alk\_PHA\_DGB\_NO2}$
	67	Anoxic glycogen production (on NO_2^-)					$Y_{NOx\_Gly,DGB}$	$-Y_{NOx\_Gly,DGB}$		$i_{N\_XBio}*Y_{X\_Gly,DGB,Ax}$	$i_{P\_XBio}*Y_{X\_Gly,DGB,Ax}$	$-Y_{X\_Gly,DGB,Ax}$		1	$-Y_{Alk\_Gly\_DGB\_NO2}$
	68	Anoxic maintenance (on NO_2^-)					$-m_{DGB,NOx}$	$m_{DGB,NOx}$		i_{N_XBio}	i_{P_XBio}	-1			$Y_{Alk\_m\_DGB\_NO2}$
N2O red. processes DGB	69	Anoxic PHA degradation (on N_2O)						$-Y_{N2O\_PHA\_DGB}$	$Y_{N2O\_PHA\_DGB}$	$-i_{N\_XBio}*Y_{PHA\_X\_DGB\_Ax}$	$-i\__{P\_XBio}*Y_{PHA\_X\_DGB\_Ax}$	$Y_{PHA\_X\_DGB\_Ax}$	-1		$-Y_{Alk\_PHA\_DGB\_N2O}$
	70	Anoxic glycogen production (on N_2O)						$Y_{N2O\_Gly\_DGB}$	$-Y_{N2O\_Gly\_DGB}$	$i_{N\_XBio}*Y_{X\_Gly\_DGB\_Ax}$	$i\__{P\_XBio}*Y_{X\_Gly\_DGB\_Ax}$	$-Y_{X\_Gly\_DGB\_Ax}$		1	$Y_{Alk\_Gly\_DGB\_N2O}$
	71	Anoxic maintenance (on N_2O)						$-m\__{DGB\_N2O}$	$m\__{DGB\_N2O}$	$i\__{N\_XBio}$	$i\__{P\_XBio}$	-1			$Y_{Alk\_m\_DGB\_N2O}$
		COMPOSITION MATRIX	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{GAO}	X_{GAO_PHA}	X_{GAO_Gly}	S_{Alk}
COD		gCOD/g	1	1	-1	i_{COD_NO3}	i_{COD_NO2}	i_{COD_N2O}	i_{COD_N2}			1	1	1	
Nitrogen		gN/g				1	1	1	1	1		i_{N_XBio}			
Phosphorus		gP/g									1	i_{P_XBio}			
Ionic charge		charge/g	i_{Charge_Ac}	i_{Charge_Pr}		i_{Charge_NO3}	i_{Charge_NO2}			i_{Charge_NHx}	i_{Charge_PO4}				-1

pH dependent variables

A.7 Balances of COD, N, P and chargues

Table 32: Balances of COD, N, P and charges for OHOs and ANOs (AOO and NOO)

#	Hydrolysis processes	S_F	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	S_{Alk}	X_{OHO}	X_{AOO}	X_{NOO}	XC_B	X_U	S_U	Σ (g COD)	Σ (g N)	Σ (g P)	Σ (charges)
1	Aerobic hydrolysis	1								0.000	0.000	0.000				-1		0	0.000	0.000	0.000	0.000
2	Anoxic hydrolysis	1								0.000	0.000	0.000				-1		0	0.000	0.000	0.000	0.000
3	Anaerobic hydrolysis	1								0.000	0.000	0.000				-1		0	0.000	0.000	0.000	0.000
#	Ordinary Heterotrophic Organisms (OHO)	S_F	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	S_{Alk}	X_{OHO}	X_{AOO}	X_{NOO}	XC_B	X_U	S_U	Σ (g COD)	Σ (g N)	Σ (g P)	Σ (charges)
4	Aerobic growth of X_{OHO} on S_F	-1.587			-0.587					-0.030	0.003	-0.002	1						0.000	0.000	0.000	0.000
5	Aerobic growth of X_{OHO} on S_{VFA}		-0.952	-0.635	-0.587					-0.078	-0.013	0.016	1						0.000	0.000	0.000	0.000
6	Anoxic growth of X_{OHO} on S_F (using NO_3)	-1.493				-0.431	0.431			-0.033	0.002	-0.002	1						0.000	0.000	0.000	0.000
7	Anoxic growth of X_{OHO} on S_F (using NO_2)	-1.493					-0.431	0.431		-0.033	0.002	0.028	1						0.000	0.000	0.000	0.000
8	Anoxic growth of X_{OHO} on S_F (using N_2O)	-1.493						-0.862	0.862	-0.033	0.002	-0.002	1						0.000	0.000	0.000	0.000
9	Anoxic growth of X_{OHO} on S_{VFA} (using NO_3)		-0.896	-0.597		-0.431	0.431			-0.078	-0.013	0.014	1						0.000	0.000	0.000	0.000
10	Anoxic growth of X_{OHO} on S_{VFA} (using NO_2)		-0.896	-0.597			-0.431	0.431		-0.078	-0.013	0.045	1						0.000	0.000	0.000	0.000
11	Anoxic growth of X_{OHO} on S_{VFA} (using N_2O)		-0.896	-0.597				-0.862	0.862	-0.078	-0.013	0.014	1						0.000	0.000	0.000	0.000
12	Fermentation	-1	0.600	0.400						0.030	0.010	-0.011							0.000	0.000	0.000	0.000
13	Lysis									0.048	0.003	0.003	-1			0.900	0.100		0.000	0.000	0.000	0.000
#	Nitrification (AOO and NOO)	S_F	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	S_{Alk}	X_{OHO}	X_{AOO}	X_{NOO}	XC_B	X_U	S_U	Σ (g COD)	Σ (g N)	Σ (g P)	Σ (charges)
14	Aerobic growth of X_{ANO} on NHx				-16.143		5.000			-5.078	-0.013	-0.719		1					0.000	0.000	0.000	0.000
15	Aerobic growth of X_{NOO} on NO_2				-21.857	20.000	-20.000			-0.078	-0.013	-0.005			1				0.000	0.000	0.000	0.000
16	Lysis									0.048	0.003	0.003		-1		0.900	0.100		0.000	0.000	0.000	0.000

COMPOSITION MATRIX	UNITS	S_F	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	S_{Alk}	X_{OHO}	X_{AOO}	X_{NOO}	XC_B	X_U	S_U
Chemical Organic Demand	gCOD/g	1	1	1	-1	-4.571	-3.429	-2.286	-1.714				1	1	1	1	1	1
Nitrogen	gN/g	0.030				1	1	1	1	1			0.078	0.078	0.078	0.030	0.030	0.010
Phosphorus	gP/g	0.010									1		0.013	0.013	0.013	0.010	0.010	0.000
Ionic charge	carga/g		-0.016	-0.009		-0.071	-0.071			0.071	-0.048	-1						

Table 33: Balances of COD, N, P and charges for PAOs (PAOI and PAOII)

		Accumulibacter type I (PAO I)	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{PAOI}	X_{PAOI_PHA}	X_{PAOI_Gly}	X_{PAOI_PP}	S_{Alk}	Σ (g COD)	Σ (g N)	Σ (g P)	Σ (charges)
Anaerobic processes DPAO	17	Anaerobic acetate uptake	-1								0.387		1.496	-0.500	-0.387	0.009	-0.004	0.000	0.000	0.000
	18	Anaerobic propionate uptake		-1							0.249		1.283	-0.283	-0.249	0.005	0.000	0.000	0.000	0.000
	19	Anaerobic maintenance (PP)									1				-1	-0.016	0.000	0.000	0.000	0.000
Aerobic processes DPAO	20	Aerobic PHA degradation			-0.246					-0.059	-0.010	0.754	-1			-0.004	0.000	0.000	0.000	0.000
	21	Aerobic glycogen production			0.111					0.069	0.012	-0.889		1		0.004	0.000	0.000	0.000	0.000
	22	Aerobic Poly-P formation			-0.236					0.018	-0.997	-0.236			1	0.017	0.000	0.000	0.000	0.000
	23	Aerobic maintenance			-1					0.078	0.013	-1				0.005	0.000	0.000	0.000	0.000
NO3 reduction processes DPAO	24	Anoxic PHA degradation (on NO3)				-0.341	0.341			-0.048	-0.008	0.610	-1			-0.003	0.000	0.000	0.000	0.000
	25	Anoxic glycogen production (on NO3)				0.166	-0.166			0.063	0.011	-0.810		1		0.004	0.000	0.000	0.000	0.000
	26	Anoxic Poly-P formation (on NO3)				-0.307	0.307			0.027	-0.995	-0.351			1	0.018	0.000	0.000	0.000	0.000
	27	Anoxic maintenance (on NO3)				-0.875	0.875			0.078	0.013	-1				0.005	0.000	0.000	0.000	0.000
NO2 reduction processes DPAO	28	Anoxic PHA degradation (on NO2)					-0.341	0.341		-0.048	-0.008	0.610	-1			0.021	0.000	0.000	0.000	0.000
	29	Anoxic glycogen production (on NO2)					0.166	-0.166		0.063	0.011	-0.810		1		-0.008	0.000	0.000	0.000	0.000
	30	Anoxic Poly-P formation (on NO2)					-0.307	0.307		0.027	-0.995	-0.351			1	0.040	0.000	0.000	0.000	0.000
	31	Anoxic maintenance (on NO2)					-0.875	0.875		0.078	0.013	-1				0.067	0.000	0.000	0.000	0.000
N2O reduction processes DPAO	32	Anoxic PHA degradation (on N2O)						-0.682	0.682	-0.048	-0.008	0.610	-1			-0.003	0.000	0.000	0.000	0.000
	33	Anoxic glycogen production (on N2O)						0.333	-0.333	0.063	0.011	-0.810		1		0.004	0.000	0.000	0.000	0.000
	34	Anoxic Poly-P formation (on N2O)						-0.614	0.614	0.027	-0.995	-0.351			1	0.018	0.000	0.000	0.000	0.000
	35	Anoxic maintenance (on N2O)						-1.750	1.750	0.078	0.013	-1				0.005	0.000	0.000	0.000	0.000
	#	Accumulibacter type II (PAO II)	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{PAOII}	X_{PAOII_PHA}	X_{PAOII_Gly}	X_{PAOII_PP}	S_{Alk}	Σ (g COD)	Σ (g N)	Σ (g P)	Σ (charges)
Anaerobic processes PAO	36	Anaerobic acetate uptake	-1								0.387		1.496	-0.500	-0.387	0.009	-0.004	0.000	0.000	0.000
	37	Anaerobic propionate uptake		-1							0.249		1.283	-0.283	-0.249	0.005	0.000	0.000	0.000	0.000
	38	Anaerobic maintenance (PP)									1				-1	-0.016	0.000	0.000	0.000	0.000
Aerobic processes PAO	39	Aerobic PHA degradation			-0.246					-0.059	-0.010	0.754	-1			-0.004	0.000	0.000	0.000	0.000
	40	Aerobic glycogen production			0.111					0.069	0.012	-0.889		1		0.004	0.000	0.000	0.000	0.000
	41	Aerobic Poly-P formation			-0.236					0.018	-0.997	-0.236			1	0.017	0.000	0.000	0.000	0.000
	42	Aerobic maintenance			-1					0.078	0.013	-1				0.005	0.000	0.000	0.000	0.000
NO2 reduction processes PAO	43	Anoxic PHA degradation (on NO2)					-0.341	0.341		-0.048	-0.008	0.610	-1			0.021	0.000	0.000	0.000	0.000
	44	Anoxic glycogen production (on NO2)					0.166	-0.166		0.063	0.011	-0.810		1		-0.008	0.000	0.000	0.000	0.000
	45	Anoxic Poly-P formation (on NO2)					-0.307	0.307		0.027	-0.995	-0.351			1	0.040	0.000	0.000	0.000	0.000
	46	Anoxic maintenance (on NO2)					-0.875	0.875		0.078	0.013	-1				0.067	0.000	0.000	0.000	0.000
N2O reduction processes PAO	47	Anoxic PHA degradation (on N2O)						-0.682	0.682	-0.048	-0.008	0.610	-1			-0.003	0.000	0.000	0.000	0.000
	48	Anoxic glycogen production (on N2O)						0.333	-0.333	0.063	0.011	-0.810		1		0.004	0.000	0.000	0.000	0.000
	49	Anoxic Poly-P formation (on N2O)						-0.614	0.614	0.027	-0.995	-0.351			1	0.018	0.000	0.000	0.000	0.000
	50	Anoxic maintenance (on N2O)						-1.750	1.750	0.078	0.013	-1				0.005	0.000	0.000	0.000	0.000

COMPOSITION MATRIX	Units	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{PAO}	X_{PAO_PHA}	X_{PAO_Gly}	X_{PAO_PP}	S_{Alk}
Chemical Organic Demand	gCOD/g	1	1	-1	-4.571	-3.429	-2.286	-1.714			1	1	1		
Nitrogen	gN/g				1	1	1	1	1		0.078				
Phosphorus	gP/g									1	0.013			1	
Ionic charge	charge/g	-1/64	-1/112		-1/14	-1/14			1/14	-1.5/31				-1/31	-1

pH dependent values. Displayed values at pH 7.0

Table 34: Stoichiometry and composition matrix with values for GAOs (GB and DGB)

		Competibacter (GB)	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{GB}	X_{GB_PHA}	X_{GB_Gly}	S_{Alk}	Σ (g COD)	Σ (g N)	Σ (g P)	Σ (charges)
Anaerobic processes GB	**51**	Anaerobic acetate uptake	-1										**2.168**	**-1.118**	0.016	0.050	0.000	0.000	0.000
	52	Anaerobic propionate uptake		-1									1.506	-0.574	0.009	-0.068	0.000	0.000	0.000
	53	Anaerobic maintenance											1	-1		0.000	0.000	0.000	0.000
Aerobic processes GB	**54**	Aerobic PHA degradation			-0.242					-0.059	-0.010	0.758	-1		-0.004	0.000	0.000	0.000	0.000
	55	Aerobic glycogen production			0.114					0.069	0.011	-0.886		1	0.004	0.000	0.000	0.000	0.000
	56	Aerobic maintenance			-1					0.078	0.013	-1				0.005	0.000	0.000	0.000
	#	**Denitrifying Competibacter (DGB)**	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{DGB}	X_{DGB_PHA}	X_{DGB_Gly}	S_{Alk}	Σ (g COD)	Σ (g N)	Σ (g P)	Σ (charges)
Anaerobic processes DGB	**57**	Anaerobic acetate uptake	-1										**2.168**	**-1.118**	0.016	0.050	0.000	0.000	0.000
	58	Anaerobic propionate uptake		-1									1.506	-0.574	0.009	-0.068	0.000	0.000	0.000
	59	Anaerobic maintenance											1	-1		0.000	0.000	0.000	0.000
Aerobic processes DGB	**60**	Aerobic PHA degradation			-0.242					-0.059	-0.010	0.758	-1		-0.004	0.000	0.000	0.000	0.000
	61	Aerobic glycogen production			0.114					0.069	0.011	-0.886		1	0.004	0.000	0.000	0.000	0.000
	62	Aerobic maintenance			-1					0.078	0.013	-1				0.005	0.000	0.000	0.000
NO3 red. processes DGB	**63**	Anoxic PHA degradation (on NO3)				-0.337	0.337			-0.048	-0.008	0.616	-1			-0.003	0.000	0.000	0.000
	64	Anoxic glycogen production (on NO3)				0.170	-0.170			0.063	0.010	-0.806		1		0.004	0.000	0.000	0.000
	65	Anoxic maintenance (on NO3)				-0.875	0.875			0.078	0.013	-1				0.005	0.000	0.000	0.000
NO2 red. processes DGB	**66**	Anoxic PHA degradation (on NO2)					-0.337	0.337		-0.048	-0.008	0.616	-1			0.021	0.000	0.000	0.000
	67	Anoxic glycogen production (on NO2)					0.170	-0.170		0.063	0.010	-0.806		1		-0.008	0.000	0.000	0.000
	68	Anoxic maintenance (on NO2)					-0.875	0.875		0.078	0.013	-1				0.067	0.000	0.000	0.000
N2O red. processes DGB	**69**	Anoxic PHA degradation (on N2O)						-0.673	0.673	-0.048	-0.008	0.616	-1			-0.003	0.000	0.000	0.000
	70	Anoxic glycogen production (on N2O)						0.339	-0.339	0.063	0.010	-0.806		1		0.004	0.000	0.000	0.000
	71	Anoxic maintenance (on N2O)						-1.750	1.750	0.078	0.013	-1				0.005	0.000	0.000	0.000

COMPOSITION MATRIX	Units	S_{Ac}	S_{Pr}	S_{O2}	S_{NO3}	S_{NO2}	S_{N2O}	S_{N2}	S_{NHx}	S_{PO4}	X_{GAO}	X_{GAO_PHA}	X_{GAO_Gly}	S_{Alk}
Chemical Organic Demand	gCOD/g	1	1	-1	-4.571	-3.429	-2.286	-1.714			1	1	1	
Nitrogen	gN/g				1	1	1	1	1		0.078			
Phosphorus	gP/g									1	0.013			1
Ionic charge	charge/g	-1/64	-1/112		-1/14	-1/14			1/14	-1.5/31				-1/31

pH dependent values. Displayed values at pH 7.0

A.8 Kinetic expressions

Table 35: Kinetic expressions for hydrolysis

	Hydrolysis	Kinetic rate equation	Switching functions (on/off)
1	Aerobic hydrolysis	$q_{XCB\_SB,hyd} \cdot \frac{\frac{XC_B}{X_{OHO}+X_{PAO}+X_{GAO}}}{K_{XCB,hyd}+\frac{XC_B}{X_{OHO}+X_{PAO}+X_{GAO}}} \cdot (X_{OHO}+X_{PAO}+X_{GAO})$	$\frac{S_{O2}}{S_{O2}+K_{O2,hyd}}$
2	Anoxic hydrolysis	$q_{XCB\_SB,hyd} \cdot \eta_{qhyd,Ax} \cdot \frac{\frac{XC_B}{X_{OHO}+X_{PAO}+X_{GAO}}}{K_{XCB,hyd}+\frac{XC_B}{X_{OHO}+X_{PAO}+X_{GAO}}} \cdot \frac{S_{NO2}+S_{NO3}}{S_{NO2}+S_{NO3}+K_{NOx,hyd}} \cdot (X_{OHO}+X_{PAO}+$	$\left[1-\frac{S_{O2}}{S_{O2}+K_{O2,hyd}}\right]$
3	Anaerobic hydrolysis	$q_{XCB\_SB,hyd} \cdot \eta_{qhyd,An} \cdot \frac{\frac{XC_B}{X_{OHO}+X_{PAO}+X_{GAO}}}{K_{XCB,hyd}+\frac{XC_B}{X_{OHO}+X_{PAO}+X_{GAO}}} \cdot (X_{OHO}+X_{PAO}+X_{GAO})$	$\left[1-\frac{S_{O2}}{S_{O2}+K_{S,O2}}\right] \cdot \left[1-\frac{S_{NO2}+S_{NO3}}{S_{NO2}+S_{NO3}+K_{NOx}}\right]$

	OHOs	Kinetic rate equation	Switching functions (on/off)
4	Aerobic growth on S_F	$\mu_{OHO,Max} \cdot \frac{S_F}{S_F+K_{S,F}} \cdot \frac{S_F}{(S_F+S_{VFA})+K_F} \cdot \frac{S_{O2}}{S_{O2}+K_{S,O2,OHO}} \cdot X_{OHO}$	$\frac{S_{PO4}}{S_{PO4}+K_{PO4,nutr}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx,nutri}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk,OHO}}$
5	Aerobic growth on S_{VFA}	$\mu_{OHO,Max} \cdot \frac{S_{VFA}}{S_{VFA}+K_{S,VFA}} \cdot \frac{S_{VFA}}{(S_F+S_{VFA})+K_F} \cdot \frac{S_{O2}}{S_{O2}+K_{S,O2,OHO}} \cdot X_{OHO}$	$\frac{S_{PO4}}{S_{PO4}+K_{PO4,nutri}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx,nutri}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk,OHO}}$
6	Anoxic growth on S_F (using NO_3^-)	$\mu_{OHO,Max} \cdot \eta_{qOHO,NO3} \cdot \frac{S_F}{S_F+K_{S,F}} \cdot \frac{S_F}{(S_F+S_{VFA})+K_F} \cdot \frac{S_{NO3}}{S_{NO3}+K_{S,NO3,OHO}} \cdot X_{OHO}$	$\left[1-\frac{S_{O2}}{S_{O2}+K_{O2,OHO,NO3}}\right] \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,nutri}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx,nutri}}$
7	Anoxic growth on S_F (using NO_2^-)	$\mu_{OHO,Max} \cdot \eta_{qOHO,NO3} \cdot \frac{S_F}{S_F+K_{S,F}} \cdot \frac{S_F}{(S_F+S_{VFA})+K_F} \cdot \frac{S_{NO2}}{S_{NO2}+K_{S,NO2,OHO}} \cdot X_{OHO}$	$\left[1-\frac{S_{O2}}{S_{O2}+K_{O2,OHO,NO2}}\right] \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,nutri}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx,nutri}}$
8	Anoxic growth on S_F (using N_2O)	$\mu_{OHO,Max} \cdot \eta_{qOHO,N2O} \cdot \frac{S_F}{S_F+K_{S,F}} \cdot \frac{S_F}{(S_F+S_{VFA})+K_F} \cdot \frac{S_{N2O}}{S_{N2O}+K_{S,N2O,OHO}} \cdot X_{OHO}$	$\left[1-\frac{S_{O2}}{S_{O2}+K_{O2,OHO,N2O}}\right] \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,nutri}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx,nutri}}$

9	Anoxic growth on S_{VFA} (using NO_3^-)	$\mu_{OHO,Max} \cdot \eta_{qOHO,NO3} \cdot \frac{S_{VFA}}{S_{VFA}+K_{S,VFA}} \cdot \frac{S_{VFA}}{(S_F+S_{VFA})+K_F} \cdot \frac{S_{NO3}}{S_{NO3}+K_{S,NO3,OHO}} \cdot X_{OHO}$	$\left[1-\frac{S_{O2}}{S_{O2}+K_{O2,OHO,NO3}}\right] \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,nutri}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx,nutri}}$
10	Anoxic growth on S_{VFA} (using NO_2^-)	$\mu_{OHO,Max} \cdot \eta_{qOHO,NO2} \cdot \frac{S_{VFA}}{S_{VFA}+K_{S,VFA}} \cdot \frac{S_{VFA}}{(S_F+S_{VFA})+K_F} \cdot \frac{S_{NO2}}{S_{NO2}+K_{S,NO2,OHO}} \cdot X_{OHO}$	$\left[1-\frac{S_{O2}}{S_{O2}+K_{O2,OHO,NO2}}\right] \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,nutri}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx,nutri}}$
11	Anoxic growth on S_{VFA} (using N_2O)	$\mu_{OHO,Max} \cdot \eta_{qOHO,N2O} \cdot \frac{S_{VFA}}{S_{VFA}+K_{S,VFA}} \cdot \frac{S_{VFA}}{(S_F+S_{VFA})+K_F} \cdot \frac{S_{N2O}}{S_{N2O}+K_{S,N2O,OHO}} \cdot X_{OHO}$	$\left[1-\frac{S_{O2}}{S_{O2}+K_{O2,OHO,N2O}}\right] \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,nutri}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx,nutri}}$
12	Fermentation of S_F	$q_{SF\_Ac,Max} \cdot \frac{S_F}{S_F+K_{S,F,fe}} \cdot X_{OHO}$	$\left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \left[1-\frac{S_{NO2}+S_{NO3}}{S_{NO2}+S_{NO3}+K_{NOx}}\right]$
13	Heterotrophic lysis	$b_{OHO} \cdot X_{OHO}$	

	Nitrification	**Kinetic rate equation**	**Switching functions (on/off)**
14	Autotrophic growth (Nitrosomonas)	$\mu_{AOO,Max} \cdot \frac{S_{NH4}}{S_{NH4}+K_{S,NH4,AOO}} \cdot \frac{S_{O2}}{S_{O2}+K_{S,O2,AOO}} \cdot X_{AOO}$	$\frac{S_{PO4}}{S_{PO4}+K_{PO4,nutri}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk,ANO}}$
15	Autotrophic growth (Nitrobacter)	$\mu_{NOO,Max} \cdot \frac{S_{NO2}}{S_{NO2}+K_{S,NO2,NOO}} \cdot \frac{S_{O2}}{S_{O2}+K_{S,O2,NOO}} \cdot X_{NOO}$	$\frac{S_{PO4}}{S_{PO4}+K_{PO4,nutri}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx,nutri}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk,ANO}}$
16	Autotrophic lysis	$b_{AOO} \cdot X_{AOO}$	

	PAOI	**Kinetic rate equation**	**Switching functions (on/off)**
17	Anaerobic acetate uptake	$q_{PAO,Ac\_PHA} \cdot \frac{S_{Ac}}{S_{Ac}+K_{S,Ac}} \cdot \frac{S_{Ac}}{(S_{Ac}+S_{Pr})+K_{VFA}} \cdot X_{PAOI}$	$\frac{X_{PAOI,PP}}{X_{PAOI,PP}+K_{PP}} \cdot \frac{X_{PAOI,Gly}}{X_{PAOI,Gly}+K_{Gly}} \cdot \frac{f_{PHA,max}-f_{PAOI,PHA}}{f_{PHA,max}-f_{PAOI,PHA}+K_{fPHA}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \left[1-\frac{S_{NO3}}{S_{NO3}+K_{NO3}}\right] \cdot \left[1-\frac{S_{NO2}}{S_{NO2}+K_{NO2}}\right]$
18	Anaerobic propionate uptake	$q_{PAO,Pr\_PHA} \cdot \frac{S_{Pr}}{S_{Pr}+K_{S,Pr}} \cdot \frac{S_{Pr}}{(S_{Ac}+S_{Pr})+K_{VFA}} \cdot X_{PAOI}$	$\frac{X_{PAOI,PP}}{X_{PAOI,PP}+K_{PP}} \cdot \frac{X_{PAOI,Gly}}{X_{PAOI,Gly}+K_{Gly}} \cdot \frac{f_{PHA,max}-f_{PAOI,PHA}}{f_{PHA,max}-f_{PAOI,PHA}+K_{fPHA}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \left[1-\frac{S_{NO3}}{S_{NO3}+K_{NO3}}\right] \cdot \left[1-\frac{S_{NO2}}{S_{NO2}+K_{NO2}}\right]$
19	Anaerobic maintenance (PP)	$m_{PAO,PP,An} \cdot X_{PAOI}$	$\frac{X_{PAOI,PP}}{X_{PAOI,PP}+K_{PP}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \left[1-\frac{S_{NO3}}{S_{NO3}+K_{NO3}}\right] \cdot \left[1-\frac{S_{NO2}}{S_{NO2}+K_{NO2}}\right]$

20	Aerobic PHA degradation	$q_{PAO,PHA,Ox} \cdot f_{PAOI,PHA}^{2/3} \cdot X_{PAOI}$	$\frac{f_{PAOI,PHA}}{f_{PAOI,PHA}+K_{fPHA}} \cdot \frac{S_{O2}}{S_{O2}+K_{O2}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk}}$
21	Aerobic glycogen production	$q_{PAO,GLY,Ox} \cdot f_{PAOI,PHA}^{2/3} \cdot \frac{1}{f_{PAOI,GLY}} \cdot X_{PAOI}$	$\frac{f_{PAO,Gly,\max}-f_{PAOI,Gly}}{f_{PAO,Gly,\max}-f_{PAOI,Gly}+K_{Gly}} \cdot \frac{X_{PAOI,PHA}}{X_{PAOI,PHA}+K_{PHA}} \cdot \frac{S_{O2}}{S_{O2}+K_{O2}}$
22	Aerobic Poly-P formation	$q_{PAO,PO4\_PP,Ox} \cdot \frac{1}{f_{PAOI,PP}} \cdot X_{PAOI}$	$\frac{f_{PAO,PP,\max}-f_{PAOI,PP}}{f_{PAO,PP,\max}-f_{PAOI,PP}+K_{PP}} \cdot \frac{X_{PAOI,PHA}}{X_{PAOI,PHA}+K_{PHA}} \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,PAO}} \cdot \frac{S_{O2}}{S_{O2}+K_{O2}}$
23	Aerobic maintenance	$m_{PAO,PHA,Ox} \cdot X_{PAOI}$	$\frac{S_{O2}}{S_{O2}+K_{O2}}$
24	Anoxic PHA degradation (on NO3)	$q_{PAO,PHA,Ax} \cdot f_{PAOI,PHA}^{2/3} \cdot X_{PAOI}$	$\frac{f_{PAOI,PHA}}{f_{PAOI,PHA}+K_{fPHA}} \cdot \frac{S_{NO3}}{S_{NO3}+K_{NO3}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk}}$
25	Anoxic glycogen production (on NO3)	$q_{PAO,GLY,Ax} \cdot f_{PAOI,PHA}^{2/3} \cdot \frac{1}{f_{PAOI,GLY}} \cdot X_{PAOI}$	$\frac{f_{PAO,Gly,\max}-f_{PAOI,Gly}}{f_{PAO,Gly,\max}-f_{PAOI,Gly}+K_{Gly}} \cdot \frac{X_{PAOI,PHA}}{X_{PAOI,PHA}+K_{PHA}} \cdot \frac{S_{NO3}}{S_{NO3}+K_{NO3}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
26	Anoxic Poly-P formation (on NO3) *(Note 2)*	$q_{PAO,PO4\_PP,Ax} \cdot \frac{1}{f_{PAOI,PP}} \cdot e^{-\frac{170}{14}S_{HNO2}} \cdot X_{PAOI}$	$\frac{f_{PAO,PP,\max}-f_{PAOI,PP}}{f_{PAO,PP,\max}-f_{PAOI,PP}+K_{PP}} \cdot \frac{X_{PAOI,PHA}}{X_{PAOI,PHA}+K_{PHA}} \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,PAO}} \cdot \frac{S_{NO3}}{S_{NO3}+K_{NO3}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
27	Anoxic maintenance (on NO3)	$m_{PAO,PHA,Ax} \cdot X_{PAOI}$	$\frac{S_{NO3}}{S_{NO3}+K_{NO3}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
28	Anoxic PHA degradation (on NO2)	$q_{PAO,PHA,Ax} \cdot f_{PAOI,PHA}^{2/3} \cdot X_{PAOI}$	$\frac{f_{PAOI,PHA}}{f_{PAOI,PHA}+K_{fPHA}} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2}} \cdot \frac{S_{NO2}}{(S_{NO2}+S_{NO3})+K_{NO3,NO2}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk}}$
29	Anoxic glycogen production (on NO2)	$q_{PAO,Gly,Ax} \cdot f_{PAOI,PHA}^{2/3} \cdot \frac{1}{f_{PAOI,Gly}} \cdot X_{PAOI}$	$\frac{f_{PAO,Gly,\max}-f_{PAOI,Gly}}{f_{PAO,Gly,\max}-f_{PAOI,Gly}+K_{Gly}} \cdot \frac{X_{PAOI,PHA}}{X_{PAOI,PHA}+K_{PHA}} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2}} \cdot \frac{S_{NO2}}{(S_{NO2}+S_{NO3})+K_{NO3,NO2}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
30	Anoxic Poly-P formation (on NO2) *(Note 2)*	$q_{PAO,PO4\_PP,Ax} \cdot \frac{1}{f_{PAOI,PP}} \cdot e^{-\frac{170}{14}S_{HNO2}} \cdot X_{PAOI}$	$\frac{f_{PAO,PP,\max}-f_{PAOI,PP}}{f_{PAO,PP,\max}-f_{PAOI,PP}+K_{PP}} \cdot \frac{X_{PAOI,PHA}}{X_{PAOI,PHA}+K_{PHA}} \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,PAO}} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2}} \cdot \frac{S_{NO2}}{(S_{NO2}+S_{NO3})+K_{NO3,NO2}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
31	Anoxic maintenance (on NO2)	$m_{PAO,PHA,Ax} \cdot X_{PAOI}$	$\frac{S_{NO2}}{S_{NO2}+K_{NO2}} \cdot \frac{S_{NO2}}{(S_{NO2}+S_{NO3})+K_{NO3,NO2}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$

32	Anoxic PHA degradation (on N2O) *(Note 2)*	$q_{PAO,PHA,Ax} \cdot f_{PAOI,PHA}^{2/3} \cdot e^{-\frac{757}{14}\cdot S_{HNO2}} \cdot X_{PAOI}$	$\frac{f_{PAOI,PHA}}{f_{PAOI,PHA}+K_{fPHA}} \cdot \frac{S_{N2O}}{S_{N2O}+K_{N2O}} \cdot \frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_{NO2,N2O}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk}}$
33	Anoxic glycogen production (on N2O)	$q_{PAO,GLY,Ax} \cdot f_{PAOI,PHA}^{2/3} \cdot \frac{1}{f_{PAOI,Gly}} \cdot X_{PAOI}$	$\frac{f_{PAO,Gly,\max}-f_{PAOI,Gly}}{f_{PAO,Gly,\max}-f_{PAOI,Gly}+K_{Gly}} \cdot \frac{X_{PAOI,PHA}}{X_{PAOI,PHA}+K_{PHA}} \cdot \frac{S_{N2O}}{S_{N2O}+K_{N2O}} \cdot \frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_{NO2,N2O}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
34	Anoxic Poly-P formation (on N2O) *(Note 2)*	$q_{PAO,PO4\_PP,Ax} \cdot \frac{1}{f_{PAOI,PP}} \cdot e^{-\frac{170}{14}\cdot S_{HNO2}} \cdot X_{PAOI}$	$\frac{f_{PAO,PP,\max}-f_{PAOI,PP}}{f_{PAO,PP,\max}-f_{PAOI,PP}+K_{S,PP}} \cdot \frac{X_{PAOI,PHA}}{X_{PAOI,PHA}+K_{S,PHA}} \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,PAO}} \cdot \frac{S_{N2O}}{S_{N2O}+K_{S,N2O}} \cdot \frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_{NO2,N2O}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{S,O2}}\right]$
35	Anoxic maintenance (on N2O)	$m_{PAOI,PHA,Ax} \cdot X_{PAOI}$	$\frac{S_{N2O}}{S_{N2O}+K_{N2O}} \cdot \frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_{NO2,N2O}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$

	PAOII	Kinetic rate equation	Switching functions (on/off)
36	Anaerobic acetate uptake	$q_{PAO,Ac\_PHA} \cdot \frac{S_{Ac}}{S_{Ac}+K_{S,Ac}} \cdot \frac{S_{Ac}}{(S_{Ac}+S_{Pr})+K_{VFA}} \cdot X_{PAOII}$	$\frac{X_{PAOII,PP}}{X_{PAOII,PP}+K_{S,PP}} \cdot \frac{X_{PAOII,Gly}}{X_{PAOII,Gly}+K_{Gly}} \cdot \frac{f_{PHA,\max}-f_{PAOII,PHA}}{f_{PHA,\max}-f_{PAOII,PHA}+K_{fPHA}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \left[1-\frac{S_{NO2}}{S_{NO2}+K_{NO2}}\right]$
37	Anaerobic propionate uptake	$q_{PAO,Pr\_PHA} \cdot \frac{S_{Pr}}{S_{Pr}+K_{S,Pr}} \cdot \frac{S_{Pr}}{(S_{Ac}+S_{Pr})+K_{VFA}} \cdot X_{PAOII}$	$\frac{X_{PAOII,PP}}{X_{PAOII,PP}+K_{PP}} \cdot \frac{X_{PAOII,Gly}}{X_{PAOII,Gly}+K_{Gly}} \cdot \frac{f_{PHA,\max}-f_{PAOII,PHA}}{f_{PHA,\max}-f_{PAOII,PHA}+K_{fPHA}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \left[1-\frac{S_{NO2}}{S_{NO2}+K_{NO2}}\right]$
38	Anaerobic maintenance (Poly-P)	$m_{PAO,PP,An} \cdot X_{PAOII}$	$\frac{X_{PAOII,PP}}{X_{PAOII,PP}+K_{PP}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \left[1-\frac{S_{NO2}}{S_{NO2}+K_{NO2}}\right]$
39	Aerobic PHA degradation	$q_{PAO,PHA,Ox} \cdot f_{PAOII,PHA}^{2/3} \cdot X_{PAOII}$	$\frac{f_{PAOII,PHA}}{f_{PAOII,PHA}+K_{fPHA}} \cdot \frac{S_{O2}}{S_{O2}+K_{O2}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk}}$
40	Aerobic glycogen production	$q_{PAO,Gly,Ox} \cdot f_{PAOII,PHA}^{2/3} \cdot \frac{1}{f_{PAOII,Gly}} \cdot X_{PAOII}$	$\frac{f_{PAO,Gly,\max}-f_{PAOII,Gly}}{f_{PAO,Gly,\max}-f_{PAOII,Gly}+K_{Gly}} \cdot \frac{X_{PAOII,PHA}}{X_{PAOII,PHA}+K_{PHA}} \cdot \frac{S_{O2}}{S_{O2}+K_{O2}}$
41	Aerobic Poly-P formation	$q_{PAO,PO4\_PP,Ox} \cdot \frac{1}{f_{PAOII,PP}} \cdot X_{PAOII}$	$\frac{f_{PAO,PP,\max}-f_{PAOII,PP}}{f_{PAO,PP,\max}-f_{PAOII,PP}+K_{PP}} \cdot \frac{X_{PAOII,PHA}}{X_{PAOII,PHA}+K_{PHA}} \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,PAO}} \cdot \frac{S_{O2}}{S_{O2}+K_{O2}}$
42	Aerobic maintenance	$m_{PAO,PHA,Ox} \cdot X_{PAOII}$	$\frac{S_{O2}}{S_{O2}+K_{O2}}$

43	Anoxic PHA degradation (on NO2)	$q_{PAO,PHA,Ax} \cdot f_{PAOII,PHA}^{2/3} \cdot X_{PAOII}$	$\frac{f_{PAOII,PHA}}{f_{PAOII,PHA}+K_{fPHA}} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk}}$
44	Anoxic glycogen production (on NO2)	$q_{PAO,Gly,Ax} \cdot f_{PAOII,PHA}^{2/3} \cdot \frac{1}{f_{PAOII,Gly}} \cdot X_{PAOII}$	$\frac{f_{PAO,Gly,\max}-f_{PAOII,Gly}}{f_{PAO,Gly,\max}-f_{PAOII,Gly}+K_{Gly}} \cdot \frac{X_{PAOII,PHA}}{X_{PAOII,PHA}+K_{PHA}} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
45	Anoxic Poly-P formation (on NO2) *(Note 2)*	$q_{PAO,PO4\_PP,Ax} \cdot \frac{1}{f_{PAOII,PP}} \cdot e^{-\frac{170}{14}\cdot S_{HNO2}} \cdot X_{PAOII}$	$\frac{f_{PAO,PP,\max}-f_{PAOII,PP}}{f_{PAO,PP,\max}-f_{PAOII,PP}+K_{PP}} \cdot \frac{X_{PAOII,PHA}}{X_{PAOII,PHA}+K_{PHA}} \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,PAO}} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
46	Anoxic maintenance (on NO2)	$m_{PAO,PHA,Ax} \cdot X_{PAOII}$	$\frac{S_{NO2}}{S_{NO2}+K_{NO2}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
47	Anoxic PHA degradation (on N2O) *(Note 2)*	$q_{PAO,PHA,Ax} \cdot f_{PAOII,PHA}^{2/3} \cdot e^{-\frac{757.7}{14}\cdot S_{HNO2}} \cdot X_{PAOII}$	$\frac{f_{PAOII,PHA}}{f_{PAOII,PHA}+K_{fPHA}} \cdot \frac{S_{N2O}}{S_{N2O}+K_{N2O}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{S,O2}}\right] \cdot \frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_{NO2,N2O}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{}}$
48	Anoxic glycogen production (on N2O)	$q_{PAO,Gly,Ax} \cdot f_{PAOII,PHA}^{2/3} \cdot \frac{1}{f_{PAOII,Gly}} \cdot X_{PAOII}$	$\frac{f_{PAO,Gly,\max}-f_{PAOII,Gly}}{f_{PAO,Gly,\max}-f_{PAOII,Gly}+K_{Gly}} \cdot \frac{X_{PAOII,PHA}}{X_{PAOII,PHA}+K_{PHA}} \cdot \frac{S_{N2O}}{S_{N2O}+K_{N2O}} \cdot \frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_{NO2,N2O}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
49	Anoxic Poly-P formation (on N2O) *(Note 2)*	$q_{PAO,PO4\_PP,Ax} \cdot \frac{1}{f_{PAOII,PP}} \cdot e^{-\frac{170}{14}\cdot S_{HNO2}} \cdot X_{PAOII}$	$\frac{f_{PAO,PP,\max}-f_{PAOII,PP}}{f_{PAO,PP,\max}-f_{PAOII,PP}+K_{PP}} \cdot \frac{X_{PAOII,PHA}}{X_{PAOII,PHA}+K_{PHA}} \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,PAO}} \cdot \frac{S_{N2O}}{S_{N2O}+K_{N2O}} \cdot \frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_{NO2,N2O}} \cdot \left[1-\frac{}{S_{O2}}\right.$
50	Anoxic maintenance (on N2O)	$m_{PAO,PHA,Ax} \cdot X_{PAOII}$	$\frac{S_{N2O}}{S_{N2O}+K_{N2O}} \cdot \frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_{NO2,N2O}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$

	GB	Kinetic rate equation	Switching functions (on/off)
51	Anaerobic acetate uptake *(Note 1)*	$q_{GAO,Ac\_PHA} \cdot \frac{S_{Ac}}{S_{Ac}+K_{S,Ac}} \cdot X_{GB}$	$\frac{S_{Ac}}{(S_{Ac}+S_{Pr})+K_{VFA}} \cdot \frac{X_{GB,Gly}}{X_{GB,Gly}+K_{Gly}} \cdot \frac{f_{PHA,\max}-f_{GB,PHA}}{f_{PHA,\max}-f_{GB,PHA}+K_{fPHA}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
52	Anaerobic propionate uptake *(Note 1)*	$q_{GAO,Pr\_PHA} \cdot \frac{S_{Pr}}{S_{Pr}+K_{S,Pr}} \cdot X_{GB}$	$\frac{S_{Pr}}{(S_{Ac}+S_{Pr})+K_{VFA}} \cdot \frac{X_{GB,Gly}}{X_{GB,Gly}+K_{Gly}} \cdot \frac{f_{PHA,\max}-f_{GB,PHA}}{f_{PHA,\max}-f_{GB,PHA}+K_{fPHA}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
53	Anaerobic maintenance	$m_{GAO,Gly,An} \cdot X_{GB}$	$\frac{X_{GB,Gly}}{X_{GB,Gly}+K_{Gly}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$

54	Aerobic PHA degradation	$q_{GAO,PHA,Ox} \cdot f_{GB,PHA}{}^{2/3} \cdot X_{GB}$	$\frac{f_{GB,PHA}}{f_{GB,PHA}+K_{fPHA}} \cdot \frac{S_{O2}}{S_{O2}+K_{O2}} \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,GAO}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk}}$
55	Aerobic glycogen production	$q_{GAO,Gly,Ox} \cdot f_{GB,PHA}{}^{2/3} \cdot \frac{1}{f_{GB,Gly}} \cdot X_{GB}$	$\frac{f_{GB,Gly,\max}-f_{GB,Gly}}{f_{GB,GLY,\max}-f_{GB,Gly}+K_{Gly}} \cdot \frac{X_{GB,PHA}}{X_{GB,PHA}+K_{PHA}} \cdot \frac{S_{O2}}{S_{O2}+K_{O2}}$
56	Aerobic maintenance	$m_{GAO,PHA,Ox} \cdot X_{GB}$	$\frac{S_{O2}}{S_{O2}+K_{O2}}$

	DGB	Kinetic rate equation	Switching functions (on/off)
57	Anaerobic acetate uptake *(Note 1)*	$q_{GAO,Ac\_PHA} \cdot \frac{S_{Ac}}{S_{Ac}+K_{S,Ac}} \cdot X_{DGB}$	$\frac{S_{Ac}}{(S_{Ac}+S_{Pr})+K_{VFA}} \cdot \frac{X_{DGB,Gly}}{X_{DGB,Gly}+K_{Gly}} \cdot \frac{f_{PHA,\max}-f_{DGB,PHA}}{f_{PHA,\max}-f_{DGB,PHA}+K_{fPHA}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \left[1-\frac{S_{NO3}}{S_{NO3}+K_{NO3}}\right] \cdot \left[1-\frac{S_{NO2}}{S_{NO2}+K_{NO2}}\right]$
58	Anaerobic propionate uptake *(Note 1)*	$q_{GAO,Pr\_PHA} \cdot \frac{S_{Pr}}{S_{Pr}+K_{S,Pr}} \cdot X_{DGB}$	$\frac{S_{Pr}}{(S_{Ac}+S_{Pr})+K_{VFA}} \cdot \frac{X_{DGB,Gly}}{X_{DGB,Gly}+K_{Gly}} \cdot \frac{f_{PHA,\max}-f_{DGB,PHA}}{f_{PHA,\max}-f_{DGB,PHA}+K_{fPHA}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \left[1-\frac{S_{NO3}}{S_{NO3}+K_{NO3}}\right] \cdot \left[1-\frac{S_{NO2}}{S_{NO2}+K_{NO2}}\right.$
59	Anaerobic maintenance	$m_{GAO,Gly,An} \cdot X_{DGB}$	$\frac{X_{DGB,Gly}}{X_{DGB,Gly}+K_{Gly}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \left[1-\frac{S_{NO3}}{S_{NO3}+K_{NO3}}\right] \cdot \left[1-\frac{S_{NO2}}{S_{NO2}+K_{NO2}}\right]$
60	Aerobic PHA degradation	$q_{GAO,PHA,Ox} \cdot f_{DGB,PHA}{}^{2/3} \cdot X_{DGB}$	$\frac{f_{DGB,PHA}}{f_{DGB,PHA}+K_{fPHA}} \cdot \frac{S_{O2}}{S_{O2}+K_{S,O2}} \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,GAO}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk}}$
61	Aerobic glycogen production	$q_{GAO,GLY,Ox} \cdot f_{DGB,PHA}{}^{2/3} \cdot \frac{1}{f_{DGB,GLY}} \cdot X_{DGB}$	$\frac{f_{GB,Gly,\max}-f_{DGB,Gly}}{f_{GB,Gly,\max}-f_{DGB,Gly}+K_{Gly}} \cdot \frac{X_{DGB,PHA}}{X_{DGB,PHA}+K_{PHA}} \cdot \frac{S_{O2}}{S_{O2}+K_{O2}}$
62	Aerobic maintenance	$m_{GAO,PHA,Ox} \cdot X_{DGB}$	$\frac{S_{O2}}{S_{O2}+K_{O2}}$
63	Anoxic PHA degradation (on NO3)	$q_{DGB,PHA,Ax} \cdot f_{DGB,PHA}{}^{2/3} \cdot X_{DGB}$	$\frac{f_{DGB,PHA}}{f_{DGB,PHA}+K_{fPHA}} \cdot \frac{S_{NO3}}{S_{NO3}+K_{NO3}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,GAO}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx}} \cdot \frac{S_{Alk}}{S_{Alk}+K_{Alk}}$
64	Anoxic glycogen production (on NO3)	$q_{DGB,GLY,Ax} \cdot f_{DGB,PHA}{}^{2/3} \cdot \frac{1}{f_{DGB,Gly}} \cdot X_{DGB}$	$\frac{f_{GB,Gly,\max}-f_{DGB,Gly}}{f_{GB,Gly,\max}-f_{DGB,Gly}+K_{Gly}} \cdot \frac{X_{DGB,PHA}}{X_{DGB,PHA}+K_{PHA}} \cdot \frac{S_{NO3}}{S_{NO3}+K_{NO3}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
65	Anoxic maintenance (on NO3)	$m_{DGB,PHA,Ax} \cdot X_{DGB}$	$\frac{S_{NO3}}{S_{NO3}+K_{NO3}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$

66	Anoxic PHA degradation (on NO2)	$q_{DGB,PHA,Ax} \cdot f_{DGB,PHA}^{2/3} \cdot X_{DGB}$	$\frac{f_{DGB,PHA}}{f_{DGB,PHA}+K_{fPHA}} \cdot \frac{S_{NO2}}{(S_{NO2}+S_{NO3})+K_{NO3,NO2}} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,GAO}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx}} \cdot \frac{S_{Alk}}{S_{Alk}+K_A}$
67	Anoxic glycogen production (on NO2)	$q_{DGB,GLY,Ax} \cdot f_{DGB,PHA}^{2/3} \cdot \frac{1}{f_{DGB,Gly}} \cdot X_{DGB}$	$\frac{f_{GB,Gly,\max}-f_{DGB,Gly}}{f_{GB,Gly,\max}-f_{DGB,Gly}+K_{Gly}} \cdot \frac{X_{DGB,PHA}}{X_{DGB,PHA}+K_{PHA}} \cdot \frac{S_{NO2}}{(S_{NO2}+S_{NO3})+K_{NO3,NO2}} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
68	Anoxic maintenance (on NO2)	$m_{DGB,PHA,Ax} \cdot X_{DGB}$	$\frac{S_{NO2}}{(S_{NO2}+S_{NO3})+K_{NO3,NO2}} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
69	Anoxic PHA degradation (on N2O)	$q_{DGB,PHA,Ax} \cdot f_{DGB,PHA}^{2/3} \cdot X_{DGB}$	$\frac{f_{DGB,PHA}}{f_{DGB,PHA}+K_{S,fPHA}} \cdot \frac{S_{N2O}}{S_{N2O}+K_{N2O}} \cdot \frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_{NO2,N2O}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right] \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,GAO}} \cdot \frac{S_{NHx}}{S_{NHx}+K_{NHx}} \cdot \frac{S_{Alk}}{S_{Alk}+K_I}$
70	Anoxic glycogen production (on N2O)	$q_{DGB,GLY,Ax} \cdot f_{DGB,PHA}^{2/3} \cdot \frac{1}{f_{DGB,Gly}} \cdot X_{DGB}$	$\frac{f_{GB,Gly,\max}-f_{DGB,Gly}}{f_{GB,Gly,\max}-f_{DGB,Gly}+K_{Gly}} \cdot \frac{X_{DGB,PHA}}{X_{DGB,PHA}+K_{PHA}} \cdot \frac{S_{N2O}}{S_{N2O}+K_{N2O}} \cdot \frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_{NO2,N2O}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$
71	Anoxic maintenance (on N2O)	$m_{DGB,PHA,Ax} \cdot X_{DGB}$	$\frac{S_{N2O}}{S_{N2O}+K_{N2O}} \cdot \frac{S_{N2O}}{(S_{N2O}+S_{NO2})+K_{NO2,N2O}} \cdot \left[1-\frac{S_{O2}}{S_{O2}+K_{O2}}\right]$

Note 1: The kinetics of the VFA uptake for GAOs depends on pH value. See Table 26.

Note 2: The concentration of nitrous acid (S_{HNO2}) depends on nitrite concentration, temperature and pH value, as the following formula shows:

$$S_{HNO2} = \frac{S_{NO2}}{e^{-\frac{2300}{273+T}} * 10^{pH}}$$