Alternatives to Lower the Carbon Demand of Biological Nutrient Removal Processes

By

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Abstract

There are ongoing researches to develop alternatives to reduce the carbon demand of biological nutrient removal (BNR) processes. In this study the feasibility of three alternatives were investigated: 1- Simultaneous denitritation and phosphorous removal in integrated fixed-film activated sludge (IFAS) systems, 2- Hydrolysis of particulate COD (pCOD) under extended anaerobic condition, and 3- Hydrolysis/fermentation of activated sludge in sludge holding tank (SHT).

The simultaneous denitritation and phosphorous removal process (performed by denitrifying phosphorous accumulating organisms (DPAO)) uses carbon simultaneously for phosphorous and nitrogen removal and avoids the conventional pathway of nitrogen removal; thus, it could reduce the carbon demand of BNR process. The use of nitrite in this process would raise the operational concern of free nitrous acid (FNA) inhibition on biomass activity. Therefore, the feasibility of long term DPAO's activity with nitrite and the FNA inhibition was investigated in an integrated fixed film activated sludge (IFAS) system. It was found that the long term simultaneous denitritation and phosphorous removal in both suspended and attached biomass of IFAS system is feasible and the risk of FNA inhibition on BNR process is moderated by the contribution of attached biomass in the IFAS system.

The potential of hydrolysis of pCOD under extended anaerobic condition to produce additional carbon for enhanced biological phosphorous removal (EBPR) was investigated. Results showed that the potential of this alternative to enhance EBPR is limited in practice due to low rate of anaerobic hydrolysis. The currently used kinetics of anaerobic hydrolysis by commercial simulators could overestimate the EBPR efficiency.

The potential of hydrolysis/fermentation of activated sludge in SHT to produce additional carbon for BNR process was investigated. Results showed that SHT could enhance BNR efficiency if the activated sludge contains relatively high biodegradable pCOD (plants with low SRT and no primary sedimentation). The results of BioWin simulation indicated that the simulator could overestimate the potential benefit of SHT by failing to simulate the effect of starvation condition in SHT on EBPR. In operation of SHT, a relatively low retention time with high sludge load could increase its potential to enhance the BNR efficiency.

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Abbreviations

bpCOD	biodegradable particulate COD
BNR	Biological nutrient removal
BOD	Biological oxygen demand
COD	Chemical oxygen demand
DPAO	Denitrifying phosphorus accumulating organisms
DO	Dissolved oxygen
EBPR	Enhanced biological phosphorus removal
FCOD	Filtered COD
FNA	Free nitrous acid
GAO	Glycogen accumulating organisms
HRT	Hydraulic retention time
IFAS	Integrated fixed-film activated sludge
MBR	Membrane bioreactor
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
MBBR	Moving bed biofilm reactor
N	Nitrogen
NEWPCC	North End Wastewater Pollution Control Centre
OHO	Ordinary heterotrophic organisms
P	Phosphorous
pCOD	particulate COD
PAO	Phosphorus accumulation organisms
PHA	Poly-hydroxyalkanoate
PHB	Poly-hydroxybutyrate
PHV	Poly-hydroxyvalerate

rbCOD	readily biodegradable COD
RAS	Return activated sludge
SBR	Sequencing batch reactor
SHT	Sludge holding tank
SRT	Solids retention time
sCOD	Soluble COD
SEWPCC	South End Wastewater Pollution Control Centre
TN	Total nitrogen
TP	Total phosphorous
TSS	Total suspended solids
VFA	Volatile fatty acid
VSS	Volatile suspended solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant
WEWPCC	West End Wastewater Pollution Control Centre

1. Chapter 1: Introduction

1.1 Need for nutrient removal from wastewater

The elements of phosphorous and Nitrogen are vital for sustaining life on our planet. These nutrients are critical for living cells' activity and widely used in fertilizer, food processing and other human used products e.g. personal care product. After the products are used by human, these nutrients are returned in different from of compounds to the environment. The phosphorous and nitrogen that end up in wastewater could lead to eutrophication (algae and aquatic plants bloom, Hypoxia, etc.) in fresh water bodies. The phosphorous is shown to be the limiting element for algae bloom (Schindler et al., 2008). The nitrogen also serves as substrate for algae growth, but in the form of ammonium contributes to the formation of free ammonia acids (FNA) which is toxics for aquatic ecosystem. According to UNEP report (1994), 28 -54% of fresh water bodies around the world have eutrophication problem. For example, Lake Winnipeg in Manitoba, Canada is the world's tenth largest fresh water reservoir and is the one with most severe eutrophication problem among them. Over the past three decades the load of nitrogen and phosphorous to this lake has increased by 10% and 30%, respectively (Lake Winnipeg Stewardship Board, 2006).

The wastewater is one of the main point-sources of nutrient load to the environment. Therefore, the removal of phosphorous and nitrogen from wastewater is critical to protect the fresh water and its associated ecosystem located downstream of wastewater treatment plants (WWTP). According to the Canadian Council of Ministers of Canada (CCME) (2009), the wastewater treatment strategy is recommended by the council, but each province should determine the local effluent standards and nutrient removal policies. Currently three major wastewater treatment

plants are operated in City of Winnipeg, Manitoba, Canada. The West End Water Pollution Center (WEWWPC) removes both nitrogen and phosphorus, but the two others, South End Water Pollution Control Centre (SEWPCC) and North End Water Pollution Control Center (NEWPPC) are under construction to upgrade to perform nutrient removal. The licence of these WWTPs require that treatment process remove the total phosphorous and nitrogen from wastewater to below 1 and 15 mg L^{-1} (30-day rolling average), respectively (Manitoba Conservation, 2003).

1.2 Phosphorous removal

The phosphorous removal from wastewater could be performed using chemical or biological processes. In chemical phosphorous removal, salt of metals such as iron (e.g. $FeCl_3$) or aluminum (e.g. $Al_2(SO_4)_3$) are mixed with wastewater for short retention time. Through this, the orthophosphate (PO_4^{-3}) in wastewater reacts with the iron and forms the metal phosphate that has low solubility in the water and precipitates. The precipitate is removed from the wastewater using settling, flotation or filtration mechanism in solid separation units. The efficiency of chemical phosphorous removal process depends on the dosage of metal salts, the reaction conditions (pH, temperature and mixing intensity) and the concentration of phosphorous and wastewater characteristics (Parsons and Stephenson, 2004). The adsorption of particulate matter in wastewater to the flocs of precipitates also results in additional suspended solids removal from wastewater (Jenkins, 2007). The phosphorous removal through chemical precipitation is not generally favored for the sustainability of WWTPs. It is because the chemical addition in this method increases the sludge production. Besides, the chemical bound of phosphorous to the sludge makes the recovery of phosphorous (e.g. struvite precipitation for fertilizer production or its bioavailability (land use application of biosolids) difficult (Oleszkiewicz et al., 2015).

1.2.1 **Biological phosphorous removal**

Phosphorous could be removed from wastewater through biological processes in activated sludge systems. The activated sludge could remove phosphorous through two mechanisms: bacteria utilize phosphorous for their metabolic activity and cell growth; and the adsorption of particulate phosphorous to activated sludge's flocs (Henze et al., 2008). When the activated sludge is wasted from the treatment system, consequently phosphorous is removed from the wastewater. In a completely aerobic activated sludge system (Conventional activated sludge), the concentration of phosphorous in activated sludge is about 15 mg $gVSS^{-1}$ (Henze et al., 2008). The phosphorous removal efficiency that could be achieved through these mechanisms is 15-25% which is not enough to meet stringent effluent standards in municipal WWTPs (e.g. TP= 1 mg L^{-1}).

In late 1950, the process of enhanced biological phosphorous removal (EBPR) was discovered that could remove phosphorous biologically at significantly higher level compared with conventional activated sludge systems. In this method, the treatment process favors the growth of a group of organisms in activated sludge community named, poly-phosphate accumulating organisms (PAO). The PAO accumulate phosphorous in their cell in the form of poly-phosphate granules. The concentration of phosphorous in PAO's cell could increase up to 380 mg P $gVSS^{-1}$ and activated sludge could contain 60 to 150 mg P $gVSS^{-1}$ (Henze et al., 2008). Therefore, wasting EBPR sludge enables WWTPs to achieve low concentration of soluble phosphorous (about 0.5 mg PO_4^{-3}-P L^{-1}) in the effluent.

The principle of EBPR is based on PAO's dynamic under alternating anaerobic and anoxic/aerobic conditions. During anaerobic conditions (no oxygen or oxidized nitrogen is present), PAO could uptake volatile fatty acids (VFA) in the wastewater and store it in the form of poly-hydroxyalkanoates (PHA) in their cells (mainly in forms ply-hydroxybutyrate (PHB) and

poly-hydroxyvalerates (PHV)). The PAO attain the energy for this bioactivity from hydrolysis of poly-P and glycogen internal storage. The hydrolysis of poly-P causes that the phosphorous and cations of magnesium and potassium (used in cells to neutralize the negative charge of poly-P) are released to the liquid.

In next step, oxygen or nitrate/nitrite (depending on the type of treatment process) is provided. At the presence of these electron acceptors, PAO utilize PHA to refill the poly-P and glycogen storage and synthesizing new cells. As a result, the phosphorous and cations of magnesium and potassium are absorbed from the liquid. The uptake of phosphorous in this step (used for synthesizing new cells and refilling poly-P storage) is more than that was released during anaerobic condition. Thus, the net phosphorous removal is positive and phosphorous is removed from the wastewater. The simplified biochemical model of PAO in EBPR process is shown in Figure 1-1.

During anaerobic conditions in EBPR process, the ordinary heterotrophic organisms (OHO) could not utilize the available carbon because they lack the energy to perform this bioprocess (no internal energy storage such as glycogen or poly-P) (Oehmen et al., 2007). Therefore, the anaerobic condition at the beginning of treatment bioprocess gives a competitive advantage to PAO against other organisms in activated sludge system. There is another group of microorganisms, named glycogen accumulating organism (GAO) that like POA could uptake VFA. The GAO only use glycogen as an internal energy/carbon storage and cannot perform phosphorous removal. Therefore, they compete with PAO for readily biodegradable carbon in wastewater and could negatively affect the EBPR performance.

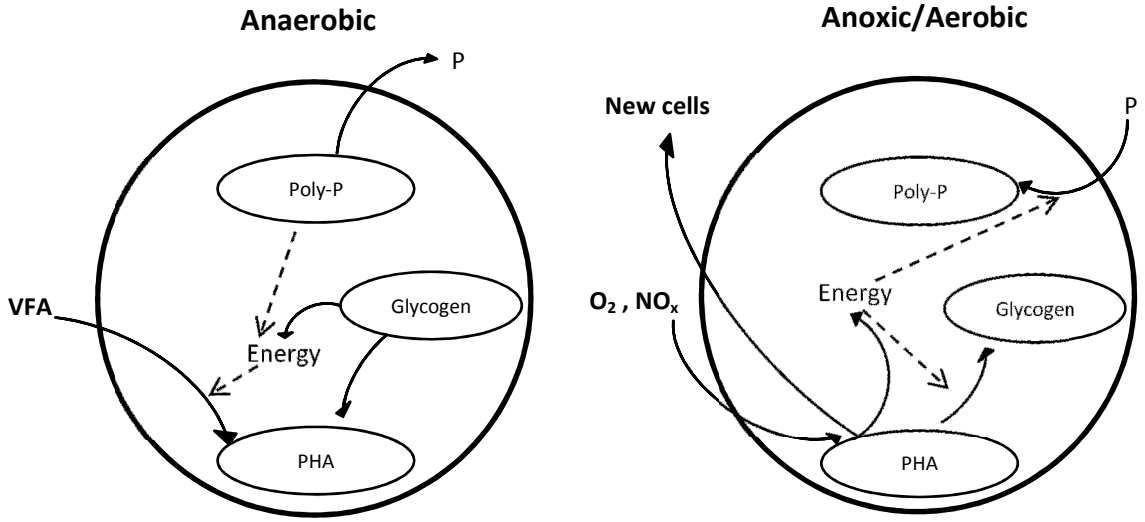

Figure 1-1 The biochemical pathway of POAs under anaerobic and aerobic/anoxic conditions (adapted from (Comeau et al., (1986))

1.2.2 **Microbiology**

The first studies on the microbiology of EBPR activated sludge was conducted using culture-dependent techniques. Based on their results, the *Acinetobacter* was identified to be the main organism responsible for phosphorous removal (Fuhs and Chen, 1975). With the development of culture-independent techniques such as fluorescence in situ hybridisation (FISH), 16S rRNA-based clone libraries or denaturing gradient gel electrophoresis (DGGE), it was found that a wide range of organisms are present in activated sludge of full-scale EBPR plants and the *Acinetobacter* is not a significant group in this community (Seviour et al., 2003; Wagner et al., 1994). Bond et al (1995) used phylogenetic analysis of 16S rRNA clone libraries to compare the community of EBPR and non-EBPR activated sludges. They found that the population of *Rhodocyclus* group from subclass 2 of the *Betaproteobacteria* was significantly higher in EBPR sludge. Bond et al. (1999) used FISH technique and reported that *Rhodocyclus*-related species

were abundant in EBPR sludge. This group of organisms proposed to be PAO are commonly abbreviated as *Candidatus Accumulibacter Phosphatis* (Hesselmann et al., 1999) or *Accumulibacter* (Oehmen et al., 2007). The studies by Hesselmann et al. (1999) and Crocetti et al. (2000) showed that *Accumulibacter* could perform the biochemical pathway of PAO. The activated sludge of different full-scale WWTPs with different process configuration were investigated by number of studies (Zilles et al., 2002a; Saunders et al., 2003; Kong et al., 2004; Gu et al., 2005; He et al., 2005; Wong et al., 2005). Their results consistently indicated that *Accumulibacter* was abundant in activated sludge community of EBPR treatment processes (4-22% of all bacteria).

The culture-independent techniques were also used to identify the key GAO in activated sludge community. Nielsen et al. (1999) used DGGE and FISH analysis to study the community of an EBPR process with low phosphorous removal efficiency (deteriorated EBPR). They observed that a group of *Gammaproteobacteria* was dominant in the system (35% of total bacteria). Crocetti et al. (2002) used the FISH probes similar to those used in Nielsen et al. (1999) and found a cluster of *Gammaproteobacteria* were dominant in a deteriorated EBPR sludge. These groups of organisms that are proposed to be GAO are commonly named *Candidatus Competibacter Phosphatis* (Nielsen et al., 1999) or *Competibacter* (Crocetti et al., 2002). The FISH probes designed for *Competibacter* has been used in several studies and the results confirmed their presence in different lab-scale EBPR (Kong et al., 2002; Oehmen et al., 2004; Zeng et al., 2003) and full scale EBPR processes (Kong et al., 2006; Saunders et al., 2003; Wong et al., 2005). Gu et al. (2005) reported that *Competibacter* was abundant in EBPR processes that exhibited low anaerobic phosphorous release to acetate uptake (GAO competition with PAO for carbon source).

Some studies showed that a group of tetrad-forming organisms (TFO) associated to *Alphaproteobacteria* were abundant in EBPR processes with poor phosphorous removal efficiency (Beer et al., 2004; Wong et al., 2004). Meyer et al. (2006) studied several full-scale WWTPs and reported that this new proposed group of GAO mostly belong to the genus *Defluviicoccus vanus* under the class of *Alphaproteobacteria*. Burow et al. (2007) studied the activated sludge community of 10 full-scale WWTPs and reported that in some plants the population of *Defluviicoccus vanus* was comparable with population of *Accumulibacter* (proposed PAO) and *Competibacter* (proposed GAO). They reported that *Defluviicoccus*-related organisms were able to take up acetate anaerobically; thus, they could compete with PAO for carbon source in EBPR systems.

1.2.3 **Effective parameters**

The composition of carbon source could affect the phosphorous removal performance by influencing the PAO-GAO competition. The acetate and propionate are the two most common forms of VFA that are available for EBPR in wastewater. The current knowledge suggest that presence of both acetate and propionate at comparable concentrations in wastewater favors the growth of PAO over GAO; therefore it could lead to stable phosphorous removal performance (Oehmen et al., 2007). It is because *Accumulibacter* performs at high substrate utilization rate with both acetate and propionate (Oehmen et al., 2004; Pijuan et al., 2004); but, the total substrate utilization rates of two proposed GAO, *Competibacter* and *Alphaproteobacteria*-GAO, are the highest when only either acetate or propionate is abundant in wastewater (Dai, 2006; Kong et al., 2006). This subject is yet to be investigated.

The pH could affect the activity of PAO and GAO differently. At higher pH, the energy that PAO require to transfer acetate into their cells increases (Liu et al., 1996; Smolders et al., 1994).

However, this does not affect the acetate uptake and PHA synthesis rates of PAO in the pH range of 6.5 to 8 (Filipe et al., 2001a). To the contrary, high pH negatively affects the metabolic activity of GAO e.g. acetate uptake rate (Filipe et al., 2001b). Under aerobic condition, the low pH conditions (below 6.5) could inhibit the PHA utilization and growth rates of PAO (Filipe et al., 2001a). Overall, it could be concluded that higher pH favors the growth of PAO over GAO in EBPR system and benefit the EBPR efficiency. This is confirmed by the results of several studies that showed when pH of anaerobic and/or aerobic conditions increased form below 7 to 7.5-8.8, the phosphorous removal efficiency improved (Bond et al., 1999; Jeon et al., 2001; Schuler and Jenkins, 2002; Serafim et al., 2002).

The temperature could also influence the PAO-GAO competition in EBPR system. The low temperatures will reduce the rate of metabolic activity of PAO and GAO; however, the study by Brdjanovic et al. (1998) showed that EBPR could be achieved at low temperature of 5 ℃. Several lab-scale researches have reported that at low temperature the efficiency of EBPR improved (Erdal et al., 2003; Panswad et al., 2003; Whang and Park, 2002). Panswad et al. (2003) and Whang and Park (2002) assessed the PAO and GAO populations in EBPR systems working at 20 ℃ and 30 ℃ and proposed that high temperature favors the growth of GAO over PAO's growth. Therefore, operating the treatment process at relatively high temperature could lead to poor EBPR performance.

There are others factors that could also affect EBPR performance. The accumulation of nitrite in the process (formation of free nitrous acid) could inhibit PAO's activity under all anaerobic/anoxic/aerobic conditions (Saito et al., 2004; Saito et al., 2008). The dissolved oxygen (DO) concentration is also reported to affect PAO and GAO populations in EBPR system. Griffiths et al. (2002) observed that poor phosphorous removal and abundance of TFO (proposed

to be GAO (Beer et al., 2004; Wong et al., 2004)) in treatment process were correlated to high DO concentration of 4-5.5 mg L^{-1}; while, PAO were present at higher population at low DO of 2.5-3 mg L^{-1}. Lemaire et al. (2006) also reported that at low DO concentration of about 0.5 mg L^{-1}, the population of Accumulibacter increased, but the population of Competibacter decreased. With regard to effect of SRT on EBPR, it has been proposed that short SRT favors PAO over GAO (Rodrigo et al., 1999; Whang and Park, 2006). These effect of DO and SRT on EBPR in full-scale WWTPs needs further investigation.

1.3 Biological nitrogen removal

The OHO, PAO and other organisms in activated sludge utilize nitrogen for their growth. The amount of nitrogen that is removed through biosynthesis is about 20% of nitrogen in wastewater (Henze et al., 2008). This is far less than that needed to meet typical nutrient removal standards of TN= 10 mg L^{-1}. There are several biological nitrogen removal processes that are used to remove the nitrogen from wastewater. In all these methods, the nitrogen in the form of ammonia or ammonium is first oxidized and then converted to nitrogen gas and released to atmosphere. Here, the principle of conventional nitrogen removal (nitrification-denitrification) is briefly explained. The oxidation of ammonium/ammonia to nitrite (nitritation, Eq. 1.1) and further to nitrate (nitratation, Eq. 1.2) is conducted by two groups of nitrifiers respectively, ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). It was initially thoughts that *Nitrosomonas* and *Nitrobacter* are the main AOBs and NOBs, respectively; but recent studies with molecular techniques have showed that several other genera could perform nitritation and nitratation (Henze et al., 2008). The nitrifiers are autotropic bacteria, thus require the inorganic carbon (e.g. CO_2 provided with aeration) for biosynthesis that is supplied by aeration in the system. The aeration also provides the oxygen that is electron acceptor for these oxidation

processes. The stoichiometric equations of these nitrification processes are presented below. The denitrification process is conducted by heterotrophic bacteria (OHO and denitrifying PAO and GAO) under anoxic conditions (no oxygen present). The electron donor for this redox bio-reaction is provided by organic carbons (e.g. COD in wastewater or acetate or methanol addition). Through this process, the nitrates is converted to N_2 and released to the atmosphere.

$$NH_4^+ + \frac{3}{2}O_2 \xrightarrow{AOB} NO_2^- + H_2O + 2H^+ \qquad [1.1]$$

$$NO_2^- + \frac{1}{2}O_2 \xrightarrow{NOB} NO_3^- \quad \text{(Nitratation)} \qquad [1.2]$$

1.3.1 **Effective parameters**

Several factors could affect the nitrification activity of activated sludge system. The nitrification process is significantly affected by wastewater compositions. It is postulated that there are substances in wastewater that have inhibitory effect on nitrifiers. Thus, it is recommended that the growth rate of nitrifiers in treatment processes is estimated using experimental data obtained with the wastewater to be treated. The growth rate of nitrifiers is highly sensitive to the ambient temperature. For every 6 ℃ reduction in temperature the growth rate of nitrifiers is reduced by half (Henze et al., 2008). This would mean the required SRT in treatment process to maintain sufficient nitrification activity should be doubled. The nitrifier are aerobes, thus the unaerated mass fraction of treatment process could affect the nitrifiers population. The effect of this factor could be realized also by the negative effect of low DO concentration on nitrifiers' kinetics (Henze et al., 2008). The DO concentration that biomass is exposed to is lower than that in the aeration basin (due to mass transfer). It is generally recommended that the DO concentration at the surface of mixed liquor in aeration basin should be maintained at 2 mg L^{-1}. The pH (in particular out of the range of 7-8) and alkalinity could significantly affect the nitrifiers' kinetics.

During nitrification, ion H^+ is produced which reduces the alkalinity of the system. The low pH and alkalinity in activated sludge systems could lead to poor nitrification activity and settling property in activated sludge systems (Jenkins et al., 2003).

The main factor that could affect the denitrification process in WWTPs is the availability of carbon source under anoxic conditions. This topic is reviewed in the following sections.

1.4 BNR process configurations

The process configuration in WWTPs has to provide the required conditions for the activity of organisms responsible for nutrient nitrogen and phosphorous removal: PAO/DPAO, OHO and nitrifiers (AOB and NOB). The process configuration is selected based on the influent characteristics (COD/N/P ratio), ambient temperature, available space and the expected nutrient removal efficiency. The arrangement of anaerobic/anoxic/aerobic basins and recycle flows in the process should enable to make the best use of carbon available in wastewater for BNR process (reducing the carbon demand of BNR process).

1.4.1 **PhoStrip® system**

The first studies by Levin and Shapiro (1965) on anaerobic phosphorous release and aerobic phosphorous uptake in activated sludge systems lead to the development of PhoStrip® system (Figure 1-2). The process configuration is consisted of an aerobic basin equipped with a side-stream anaerobic tank (stripper tank). The sludge (10 -30% of influent flow rate) form underflow of secondary clarifier is transferred to the stripper tank, where the sludge settles and the anaerobic conditions causes the phosphorous release. The stripped sludge is sent back to the aerobic basin; while, the supernatant that has high concentration of soluble phosphorous is transferred to another mixing tank. In the mixing tank, chemical (usually lime) is added and

soluble phosphorous is precipitated. In another settling tank, the precipitate (P-rich sludge) is removed and the supernatant is sent back to the primary clarifier (back to bioprocess). To promote anaerobic phosphorous release, an external carbon source such as acetate or a portion of primary influent could be sent to the stripper tank. This process combines chemical and biological phosphorous removal. The PhoStrip® process could be modified with an anoxic tank before the aerobic basin to perform biological nitrogen removal.

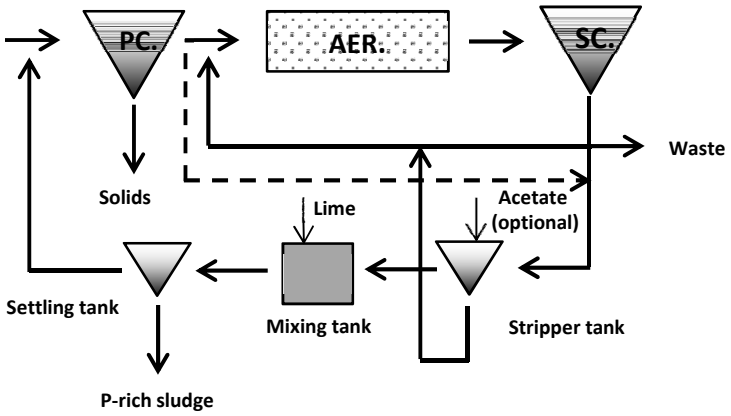

Figure 1-2 System configuration of PhoStrip®

1.4.2 **Phoredox system (A/O)**

Extensive research by Barnard (Barnard, 1974; Barnard, 1975a; Barnard, 1975b) concluded the principle of EBPR process that is the activated sludge has to be exposed to alternating anaerobic/aerobic conditions in order to achieve excessive biological phosphorous removal. The Phoredox system was developed based on this principle (Figure 1-3) and designed for WWTP that are not required to perform nitrification. The system is combination of anaerobic tank followed by an aerobic tank. In order to stop nitrification in the process, the SRT is maintained relatively short (depending on the ambient temperature).

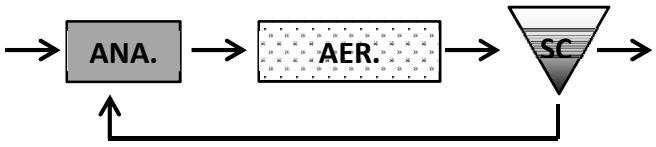

Figure 1-3 System configuration of Phoredox

1.4.3 **5- and 3-stage Modified Bardenpho**

Barnard (1976) applied the Phoredox method (anaerobic /aerobic cycle) to the 4 stage Bardenpho process (designed for nitrogen removal only) to create a BNR process, named 5-stage Bardenpho process (Figure 1-4a). The process consisted of two consecutive sets of anoxic/aerobic tanks; in the first set, the mixed liquor from the aerobic tank is recycled to the anoxic tank. The anaerobic basin is placed ahead of the aerobic/anoxic sets and influent is directed to it. It provides the required conditions to promote PAO's growth (anaerobic conditions at the presence of VFA) in the treatment process. If lower nitrogen removal efficiency is required, the second set of anoxic/aerobic tanks is removed that makes the 3-stage modified Bardenpho process (A2O) (Figure 1-4b). In modified Bardenpho processes, in particular in 3-stage with one set of anoxic/aerobic tanks, nitrate is transferred with RAS to the anaerobic tank that could negatively affect the denitrification (denitrifiers compete with PAO for carbon).

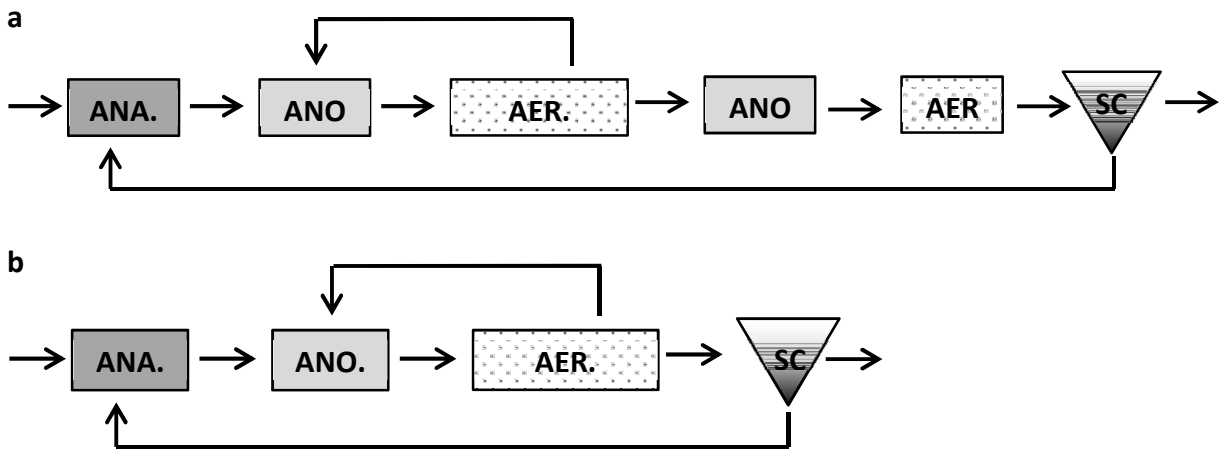

Figure 1-4 System configuration of a) 5-stage Bardenpho and b) 3-stage Bardenpho

1.4.4 **University of Cape Town (UCT) system**

Rabinowitz (1980) investigated the effective parameters on BNR efficiency of Bardenpho system. They concluded that the main obstacle to achieve reliable EBPR performance is the negative effect of nitrate in RAS transferred to anaerobic tank. They realized that reducing the RAS flow is not a proper alternative as it could affect the settleability of mixed liquor in the system. To address the negative effect of nitrate recycle to anaerobic tank, they attempted different optimization strategies that led to the development of University of Cape Town (UCT) system (Figure 1-5).

This UCT system is similar to a 3-stage Bardenpho except for the RAS flow and an additional internal recycle flow. In the UCT system, the RAS is sent back to anoxic tank and from there an additional internal recycle flow transfers mixed liquor to the anaerobic basin. As a result, the use of carbon for denitrification increases and consequently the load of nitrate to the anaerobic tank decreases. Since the sludge is sent back to t anaerobic tank through internal recycle of mixed

liquor (lower concentration of solids compared with that in RAS), a larger anaerobic tank is required (longer Anaerobic HRT).

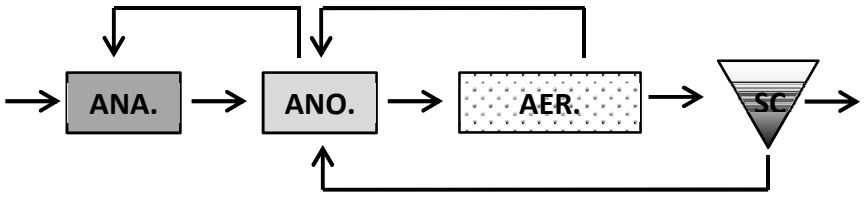

Figure 1-5 System configuration of UC

1.4.5 **Modified UCT system (MUCT)**

In UCT system, the flow of internal recycle from anoxic tank to anaerobic tank should be carefully controlled to avoid the transfer of nitrate to anaerobic tank. This is not practical in full scale operation because of typical variation of N/COD ratio in the influent. In order to further reduce the potential of nitrate recycle to anaerobic tank, the UST process was modified to have two anoxic tanks. The mixed liquor from aerobic tank is recycled to the second anoxic tank, in which most of nitrogen removal is achieved. Therefore, the modified UCT (MUCT) (Figure 1-6) will have lower load of nitrate to the first anoxic tank and consequently to the anaerobic basin. The MUCT system has two internal recycle streams which makes the process relatively complicated.

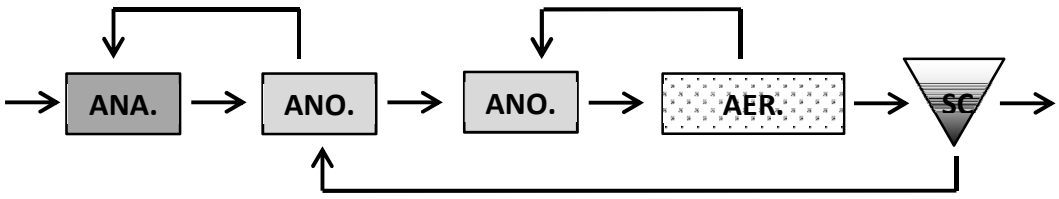

Figure 1-6 System configuration of MUCT

1.4.6 **Johannesburg (JHB) system**

The work by Osborn and Nicholls (1978) that was aimed to address the negative effect of nitrate recycle on EBPR in 5-stage Bardenpho led to the development of Johannesburg (JHB) system. The JHB system is made by repositioning of the second anoxic tank of the 5-stage Bardenpho to the RAS stream, thus, named secondary anoxic tank (Figure 1-7a). In JHB system, the load of nitrate that has to be removed in order to avoid nitrate recycle to anaerobic tank is lower than that in 5-stage Bardenpho. Therefore, the potential of EBPR deterioration because of nitrate recycle is reduced in JHB; however, lower nitrogen removal efficiency is achieved. This reason makes the JHB system more applicable for wastewater with relatively low N/COD ratio (Henze et al., 2008). The Westside (BC) (Figure 1-7b) system is similar to JHB with the option that in the Westside system the influent could be distributed between secondary anoxic tank (or pre-anoxic), anaerobic and anoxic tanks.

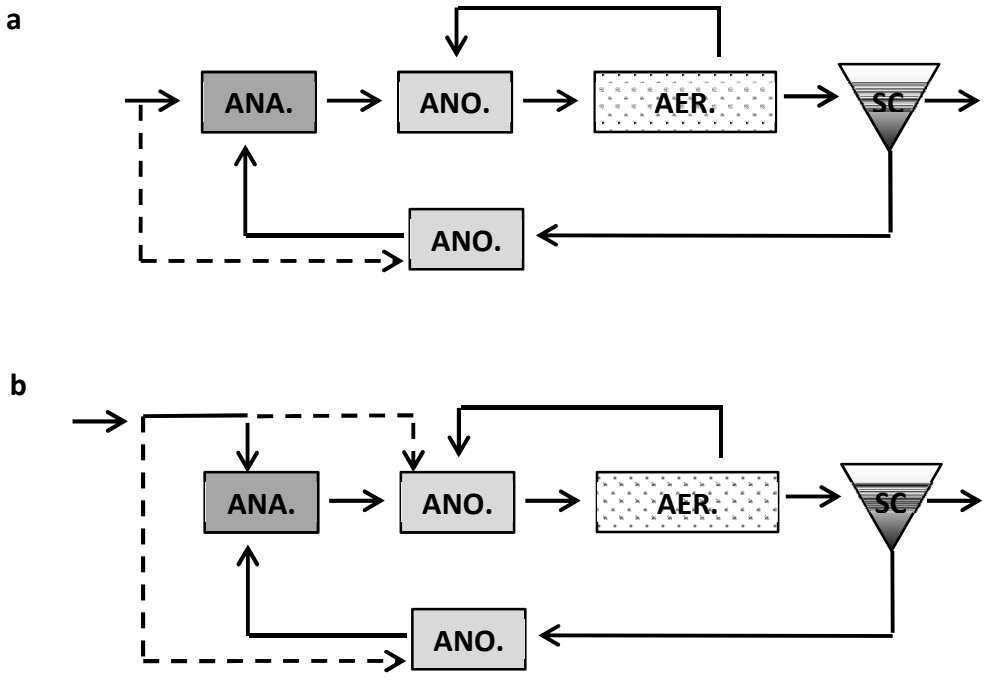

Figure 1-7 System configuration of a) JHB and b) Westside

1.4.7 **Biological-Chemical phosphorous removal (BCFS® system)**

The BCFS system (Figure 1-8) was developed based on further modification of MUCT system (Lidman et al., 1998). In BCFS system compared with MCUT, the option of chemical phosphorous removal is added to support the EBPR process. Also, a third internal recycle from aerobic tank to the first anoxic tank is included. Therefore, the denitrification capacity is improved and also the operation of second anoxic tank could be switched to aeration tank in case of peak loads. The chemical phosphorous removal is implemented by adding a stripper tank connected to anaerobic tank. The high concentration of phosphorous in anaerobic tank allows for effective use of chemicals for precipitation. The BCFS system allows for phosphorous recovery and also could improve the sludge settling property and nitrogen removal efficiency of the treatment process (Henze et al., 2008).

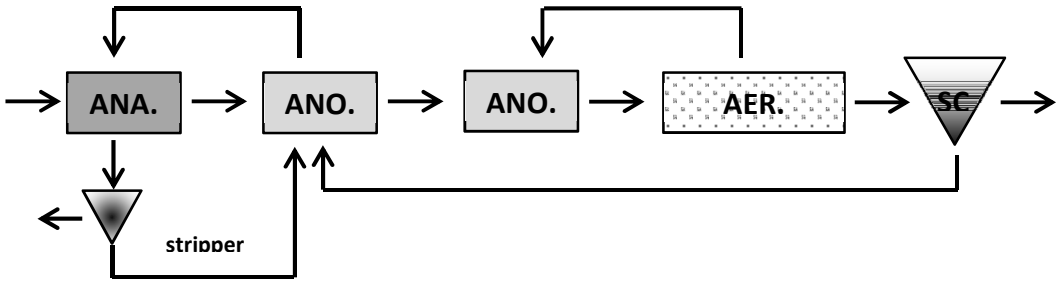

Figure 1-8 System configuration of BCFS®

1.5 Carbon deficiency in BNR processes

The most important cause that led to development of different BNR system configurations is to minimize the transfer of nitrate into anaerobic basin to secure rbCOD for PAO's activity. Both denitrifiers and PAO require carbon as electron donor to perform biological nitrogen and phosphorous removal processes, respectively. However, the amount of carbon that is available

for BNR in wastewater is not usually enough to meet the strict nutrient removal efficiencies. The efficiency of biological phosphorous removal closely correlated to the amount of VFAs that is utilized by PAO under anaerobic conditions (Randall et al., 1998). Janssen et al. (1996) reported that to remove 1 mg of phosphorous from the wastewater, 10 mg of readily biodegradable COD (rbCOD) is required. In the case of carbon deficiency, nitrate is recycled at significant load to anaerobic tank. In this conditions, denitrifiers (e.g. OHO) could outcompete POA for the available carbon and EBPR deteriorates (Morling, 2001).

To address the common problem of carbon deficiency in BNR- WWTPs, the addition of carbon source such as methanol, ethanol or acetate are widely practiced in WWTPs (Cho and Molof, 2004; Latimer et al., 2012; Puig et al., 2008). Also, the fermentation of primary sludge is commonly used to provide additional VFA for EBPR activity (Shen and Zhou, 2016). The addition of external carbon source to the treatment process results in higher operational cost (chemical supply) and sludge production of WWTPs. The cost associated to sludge treatment could make up to 60% of total operational of WWTPs. A preferable alternative could be to increase the use of non-readily biodegradable COD that exist in wastewater for BNR (e.g. activated sludge or mixed liquor hydrolysis/fermentation) or enhance the pathway of BNR to reduce its carbon demand (e.g. nitritation-denitritation and/or simultaneous denitrification and phosphorous removal).

1.6 Objective and scope

The objective of this thesis is to assess the potential of three different alternatives to reduce the carbon demand of BNR process. The three alternatives are as follows:

1. Simultaneous denitritation and phosphorous removal in integrated fixed-film activated sludge (IFAS) system

Chapter 2 investigates the feasibility of simultaneous nitrogen and phosphorous removal using nitrite as electron acceptor in IFAS system. This enhanced process is conducted by DPAO and could significantly reduce the carbon demand of BNR process. It has been already demonstrated by other researches that DPAO could utilize nitrate as electron acceptor; but, the performance of DPAO using nitrite as electron acceptor in particular in IFAS system (both suspended and attached form could contribute to EBPR) needs to be investigated. The process and the need for its investigation are further explained in Chapter 2. The experimental methods and results are described; through these, the conclusion on feasibility of DAPOs activity with nitrite in IFAS system is presented.

Chapter 3 introduces the main operational concern of the enhanced BNR process investigated in Chapter 2 that is the inhibitory effect of nitrite on DPAO/PAO in IFAS system. The methodology and results are presented. Finally, the chapter concludes on the stability of simultaneous denitritation and phosphorous removal process with regard to the FNA inhibition in the IFAS system.

2. Potential of hydrolysis of particulate COD (pCOD) under extended anaerobic condition to enhance phosphorous removal

Chapter 4 investigates the potential of extending anaerobic condition beyond typical anaerobic HRTs of 0.5 -1.5 h to increase the use of non-readily biodegradable COD in the wastewater for BNR process. The introduction in this chapter explains why this alternative requires further investigation. The performed experiments and bioprocess modeling are explained and the results and final conclusion on the potential benefit of this alternative is presented.

3. Potential of hydrolysis/fermentation of activated sludge in sludge holding tank (SHT) to enhance BNR efficiency.

Chapter 5 investigates whether use of SHT (hydrolysis/fermentation of activated sludge) in WWTP could provide additional carbon source for BNR process and enhance treatment efficiency. The chapter explains that how recent advancement in use of SHT to reduce sludge production has raised the question of the effect of SHT on WWTP's BNR performance. The experiments and bioprocess modeling in this investigation are presented. Using the results, the Chapter 5 concludes on the potential of SHT to enhance BNR efficiency of WWTPs.

2 Chapter 2: The feasibility of simultaneous denitritation and phosphorous removal in IFAS systems[1]

Abstract

Nitrite and nitrate were compared as electron acceptors to select for denitrifying phosphorous accumulating organisms (DPAO) in two integrated fixed film activated sludge (IFAS 1 and IFAS 2) systems operated as sequencing batch reactors . The bench-scale experiment lasted one year and synthetic wastewater was used as feed. During anoxic conditions 20 mg NO_3-N L^{-1} were dosed into IFAS-1 and 20 mg NO_2-N L^{-1} were dosed into IFAS-2. Long term phosphorous and ammonia removal via nitritation were achieved in both systems and both attached and suspended biomass contributed to phosphorous and ammonia removal. DPAO showed no specific adaptation to the electron acceptor as evidenced by short term switch of feeding with nitrate or nitrite. Anoxic phosphorus uptake rate was significantly higher with nitrite than with nitrate. Results showed that DPAO's activity with nitrite could be integrated into attached and suspended biomass of IFAS systems in long term operation.

[1] Published as the following citation: "Jabari P, Munz G, Oleszkiewicz J a. 2014. *Selection of denitrifying phosphorous accumulating organisms in IFAS systems: Comparison of nitrite with nitrate as an electron acceptor*. Chemosphere **109**:20–27."

2.1 Introduction

Integrated fixed film activated sludge (IFAS) systems are increasingly gaining interest in biological wastewater treatment (Christensson and Welander, 2004). The co-existence of attached and suspended sludge in the same reactor allows for higher biomass concentration and diverse solids retention times (SRT) in the same reactor and this provides wastewater treatment plants with higher volumetric treatment capacity (Ødegaard, 2006).

The advantages of IFAS systems to improve nitrification have been already widely documented. According to Randall and Sen (1996), the introduction of fixed-film media into the aerobic basin of a full-scale anoxic-aerobic system almost doubled nitrification rate; moreover, the occurrence of denitrification in the biofilm allowed for reduction of the anoxic tank volume. Stricker et al. (2009) compared nitrification performance in conventional activated sludge and IFAS systems (floating carrier material) in two full-scale parallel trains. Having similar suspended biomass concentration, IFAS system contained 50% more biomass due to attached growth which resulted in twice the treatment capacity and exhibited more stable nitrification in cold temperature.

While the advantages of IFAS technology in nitrogen removal have been demonstrated, its role in biological phosphorus removal still remains to be investigated. Pilot and full-scale tests showed that IFAS systems could sustain enhanced biological phosphorous removal (EBPR) as well (Rogalla et al., 2006). Sriwiriyarat and Randall (2005) reported successful EBPR and nitrification in an IFAS reactor; while its performance was affected by biomass distribution between media and suspended biomass and availability of carbon source in the anaerobic zone. Kim et al., (2011) compared nutrient removal performance of an IFAS system (media in the aerobic zone) and a conventional activated sludge system treating real wastewater. In both systems, high COD and phosphorous removal were obtained at SRT of the suspended biomass

($SRT_{suspended}$) of 8 d. In these studies phosphorous removal was mainly achieved by suspended biomass.

Attached biomass could be incorporated into biological phosphorous removal by applying alternating anaerobic and aerobic conditions in time rather than in space in the treatment process (Rogalla et al., 2006). This concept has been successfully applied to recover phosphorous from wastewater (Kodera et al., 2013). In conventional biological phosphorous removal, phosphorus accumulating organisms (PAO) take up carbon during anaerobic period and remove phosphorous in the following aerobic stage. Some PAO are capable of taking up phosphorous using nitrate or nitrite as an electron acceptor during anoxic conditions, so named denitrifying PAO (DPAO) (Oehmen et al., 2007). The selection of DPAO is important as simultaneous denitrification and phosphorous removal could reduce the need for carbon source, sludge production and energy consumption in biological nutrient removal processes (Kuba et al., 1996a). In particular, DPAO's activity over nitrite is of interest, since, employing partial nitrification in a nutrient removal process could reduce aeration demand and sludge production compared to full nitrification-denitrification(Peng and Zhu, 2006).

The DPAO's activity using nitrate or nitrite as an electron acceptor in suspended biomass systems has been extensively studied. Nitrate is widely accepted as substrate for anoxic phosphorous uptake (Ahn et al., 2001; Hu et al., 2003); while inhibitory effects of nitrite on anoxic and aerobic phosphorous uptake have been reported in the literature. Saito et al., (2004) reported negative effects of nitrite on anoxic and particularly on aerobic phosphorous uptake in a suspended growth system; in fact, a complete inhibition of aerobic phosphorous uptake was observed at nitrite concentration of 6 mg NO2--N L-1. Sin et al. (2008) found that nitrite could be utilized for anoxic phosphorous uptake in an anaerobic/anoxic/aerobic sequencing batch

reactor (SBR); however, at concentration of 6 mg NO2--N L-1 the aerobic phosphorous uptake was completely inhibited while anoxic phosphorous uptake dropped by 35% at the concentration of 12 mg NO2--N L-1.

On the other hand, Hu et al. (2003) reported that nitrite at the concentration of up to 115 mg N L-1 did not inhibit anoxic phosphorous uptake activity of DPAO previously acclimated to nitrate as an electron acceptor in an anaerobic/anoxic SBR. In batch studies carried out by Zhou et al. (2012), PAO from an anaerobic/aerobic SBR could utilize nitrite as an electron acceptor and the highest anoxic phosphorous uptake and denitrification rates were obtained with initial nitrite concentration of 20 mg N L-1. Vargas et al. (2011) operated an anaerobic/anoxic/aerobic SBR where DPAO could use nitrite as an electron acceptor at concentrations of more than 20 mg N L-1 during anoxic condition. Zhou et al. (2007) found a strong correlation between anoxic phosphorous uptake rate and free nitrous acid (FNA) concentration. They concluded that the different nitrite inhibitory effect reported in different studies was caused by the fact that FNA rather than nitrite is the actual inhibitor.

The objective of this study was to assess the long term behaviour of DPAO when using nitrate or nitrite as electron acceptor in an IFAS system. DPAO performance with nitrite in IFAS systems where both attached and suspended biomass are contributing to EBPR is poorly understood. Furthermore, the selected DPAO in reactors were to be characterized in terms of adaptation to nitrite and nitrate and the respective contributions of suspended and attached biomass in phosphorous and nitrogen removal.

2.2 Material and Methods

2.2.1 **Experimental set up and operation**

Two IFAS systems (3 L liquid volume each) were operated as SBR and fed with synthetic wastewater for 1 year (about 70 d for start-up and 300 d in steady state conditions). Influent composition was (all in mg L^{-1}) 240 sodium acetate, 57.5 NH_4Cl, 51 K_2HPO_4, 83 $MgSO_4$, 13 $CaCl_2$, 65 yeast extract and 65 beef extract equivalent in total to 300 mg COD L^{-1}, 30 mg TN L^{-1} and 9 mg P L^{-1}. 30% of the bulk working volume was filled with Anox Kaldnes (K1) media (specific surface area of 500 m^2 m^{-3}). Each cycle lasted 480 min and consisted of: feeding (20 min); anaerobic reaction (70 min); anoxic reaction (225 min); aerobic reaction (90 min); settling (50 min) and decanting (25 min). The reactors were operated with 12 h hydraulic residence time (HRT) and 9 d $SRT_{suspended}$. Reactors were operated at room temperature (19-21℃). The pH was not controlled and ranged from 7.2 to 8 during the whole cycle. Air feed at 1 L min^{-1} was maintained during aerobic stage in both reactors. Reactors were mixed the entire react period using a magnetic stirrer. Anoxic stage was achieved through stepwise dosing of 20 mg L^{-1} of nitrogen (60 mg NO_x-N in total) either nitrate, into IFAS-1 or nitrite into IFAS-2. Dosing was performed in eight steps every 20 min from the beginning of anoxic stage (7.5 mg NO_x-N at each step). The objective was to maintain a low concentration of electron acceptor throughout the anoxic period to limit the inhibitory effect of nitrite on biomass in the IFAS system.

2.2.2 **Kinetic tests**

When reactors reached steady state three types of kinetic tests were carried out:

1 During the SBR operation nitrate/nitrite dose was either doubled (40 mg N L^{-1}) or tripled (60 mg N L^{-1}). The objective was to study the effect of nitrate/nitrite shock loading on DPAO performance.

2 During SBR operation nitrate and nitrite dosing during anoxic phase was switched between IFAS systems with either regular or doubling the dose. The objective was to evaluate the ability of DPAO to use nitrate or nitrite facultatively.

3 Suspended sludge and media were separated and transferred into different batch reactors where the same operational conditions, nitrate or nitrite dosing amount and cycle of IFAS-1 and 2 were maintained during one SBR cycle. These kinetic tests were carried out to evaluate phosphorous and nitrogen removal by suspended and attached biomass separately. It should be noted that suspended biomass in IFAS systems could contain some attached biomass detached from the biofilm carriers. Separating biofilm carriers from suspended biomass were performed with cautious to minimize the detachment and transfer of attached biomass to suspended form.

2.2.3 **Analyses**

Nutrient removal performance of reactors was evaluated three times a week by measuring phosphate, ammonia, nitrate and nitrite at the beginning of the cycle and at the end of anaerobic, anoxic and aerobic stages. Mixed liquor concentration was measured once a week. Monitoring nitrogen compounds and phosphorous during the cycle was carried out every 2 wk. For COD, phosphate and nitrogen compounds analysis, mixed liquor samples from IFAS reactors and batch tests were immediately filtered through 0.45-µm-pore size filter. Dissolved phosphate, ammonia, nitrite and nitrate were measured by Lachat Instrument (Ontario, Canada) Quik Chem 8500, according to orthophosphate method 10-115-01-1-9 O, ammonia method 10-107-06-1-I and nitrate/nitrite 10-107-01-1-A, respectively. COD, mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were measured according to Standard Methods (APHA, 2012). To measure attached biomass concentration, 10 biofilm carriers were randomly

sampled and the attached biomass were scraped off and collected using water pressure and small brush. The produced liquid was filtered using 0.45 µm filter pore size and mass of filtrate and its volatile fraction was measured according to the method used for MLSS and MLVSS analysis, respectively. Knowing the exact number of plastic media in each reactor, the total mass and volatile fraction of biofilm in the reactors was estimated. Average MLVSS and MLVSS/MLSS ratio in IFAS-1 were 1.2 ± 0.1 g VSS L^{-1} and 0.71, respectively; these values for IFAS-2 were 1.3 ± 0.1 g VSS L^{-1} and 0.73. The attached biomass concentration and VS/TS ratio in IFAS-1 were 2.7 ± 0.3 g L^{-1} and 0.81, respectively; these values for IFAS-2 were 2.0 ± 0.3 g L-1 and 0.83. DO was monitored using WTW IQ Sensor Net, 2020 XT meter with WTW FDO 925 optical dissolved oxygen Sensor. The pH was measured by Fisher Scientific accumet, Ap115 pH portable meter with accumet pH, Ag/AgCl reference electrode.

2.3 Results and Discussion

2.3.1 **Reactors performance**

After 2 months of operation, stable phosphorous removal performance was achieved in both systems (Figure 2-1). Typical profiles of phosphorous and nitrogen compounds during reactor cycle of IFAS-1 and IFAS-2 are presented in Figure 2-2 and Figure 2-3, respectively. In the anaerobic phase, COD was rapidly depleted and phosphorous was released up to 66 ± 11 mg L^{-1} and 70 ± 7 mg L^{-1} in IFAS-1 and IFAS-2, respectively. During the following anoxic phase 25 ± 6 and 28 ± 4 mg L^{-1} of phosphorous were taken up using either nitrate or nitrite as electron acceptor in IFAS-1 and IFAS-2, respectively. In IFAS-2 nitrite was completely consumed which means that nitrite was limiting the phosphorous uptake during anoxic period. In IFAS-1 nitrate was partially utilized and about 10 mg N L^{-1} of nitrate was left at the end of anoxic stage. Remaining phosphate in both reactors was removed aerobically. The ratio of anoxic phosphorous

uptake to denitrified nitrogen (P/N) in IFAS-1 and IFAS-2 were 2.4 and 1.5, respectively. Coma et al., (2010) reported P/N ratio of 3.04 and 1.68 for granular sludge working with nitrate and nitrite, respectively.

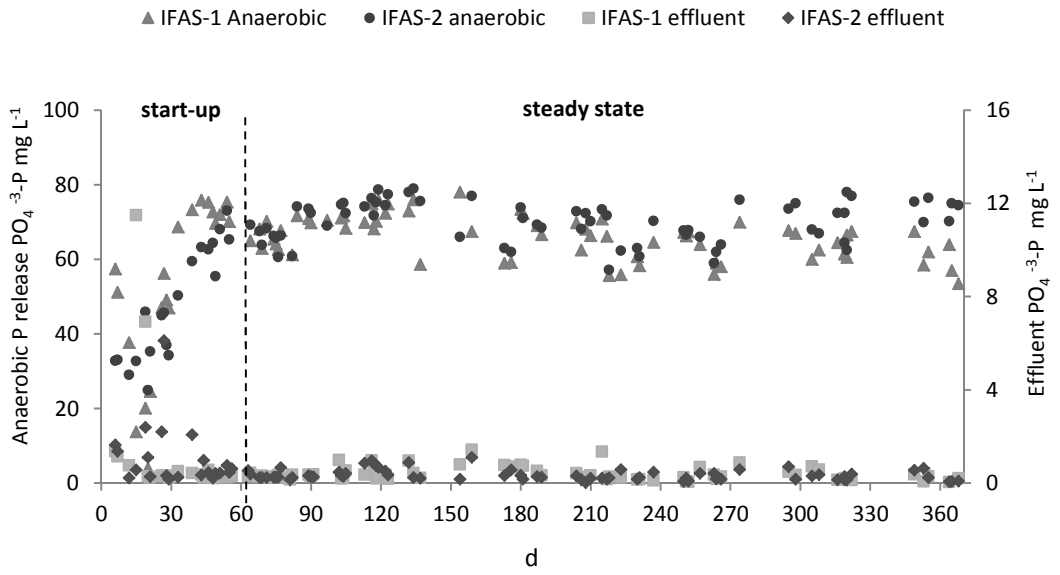

Figure 2-1 Long term phosphorus removal performance in IFAS-1 and IFAS-2

During aerobic phase, the ammonia oxidizing bacteria (AOB) nitrified ammonia completely to nitrite; nitrate was not observed in the reactors. A combination of short aerobic SRT and the competition between PAO, AOB and nitrite oxidizing bacteria (NOB) for oxygen was likely the cause and will be discussed later. A maximum specific nitritation rate (oxidation of ammonia to nitrite) in IFAS-1 and 2 were 2.3 and 1.8 mg N h^{-1} g^{-1} $VSS_{(suspended + attached)}$, respectively.

2.3.2 **Biomass adaptation to the electron acceptor**

Table 2-1 summarizes the results of kinetic tests (types 1 and 2 as per Methods) on the effect of nitrate and nitrite concentration and on biomass ability of using either nitrite or nitrate without being previously adapted.

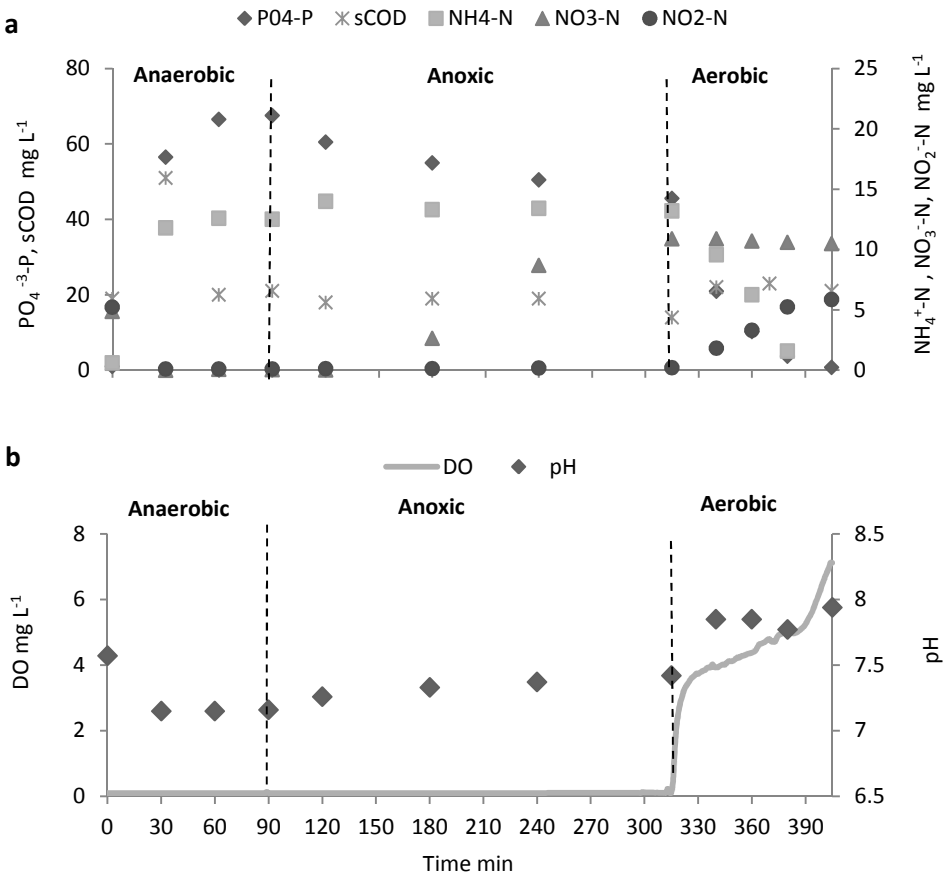

Figure 2-2 Nitrogen and phosphorus concentration (a) and DO and pH (b) profiles in IFAS-1

Similar anoxic phosphorous uptake was obtained at different nitrate dosing in IFAS-1 showing that DPAO in IFAS-1 were working at their highest capacity at regular dosing amount of 20 mg NO_3^--N L^{-1}. In IFAS-2 increasing nitrite dosing led to an increase of the anoxic phosphorus uptake; however, phosphorus concentration in the effluent increased up to 3 mg L^{-1}, exhibiting the inhibition effect of nitrite on aerobic phosphorus uptake. The average maximum specific aerobic phosphorus uptake rates in IFAS-2 during tests with 40 and 60 mg N L^{-1} of nitrite were 5.6 and 4.9 mg P h^{-1} g^{-1} $VSS_{(suspended + attached)}$, respectively, compared to 13.9 mg P h^{-1} g^{-1} $VSS_{(suspended + attached)}$ during regular operation at lower concentrations of electron acceptors.

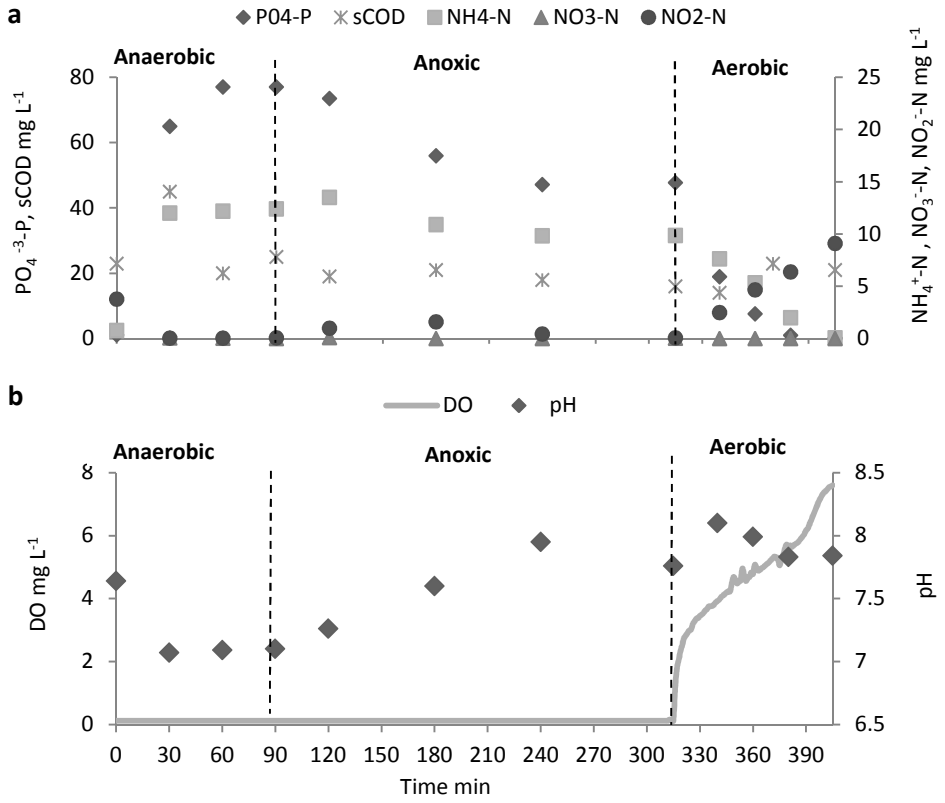

Figure 2-3 Nitrogen and phosphorus concentration (a) and DO and pH (b) profiles in IFAS-2

When nitrate and nitrite were switched between reactors with either regular or double dose (test type 2 as per Methods), DPAO in IFAS-1 that used to work with nitrate could consume all nitrite dosed into the reactor achieving respectively, 25 and 51 mg P L^{-1} anoxic uptake in 20 and 40 mg NO_2^--N L^{-1} dosing tests. On the other hand, in IFAS-2 where biomass was working under nitrite reducing environment, nitrate was partially utilized in 20 mg NO_3^--N L^{-1} dosing test with around 23 mg P L^{-1} anoxic uptake. Higher nitrate dosing in IFAS-2 (40 mg NO_3^--N L^{-1}) did not lead to higher nitrate utilization and anoxic phosphorous uptake did not improve.

In conclusion, the DPAO of both systems regardless of acclimation to different electron acceptor showed high anoxic phosphorous uptake with nitrite; on the contrary, nitrate was only partially

consumed and caused lower anoxic phosphorous uptake. Different denitrifying phosphorous removal performances with nitrate and nitrite have been reported previously (Flowers et al., 2009; He, 2008; Hu et al., 2003; Jiang et al., 2006)Based on that, DPAO have been divided into two groups (Carvalho et al., 2007; Guisasola et al., 2009): DPAO able to use oxygen, nitrate and nitrite (nitrate-DPAO) and DPAO able to use only oxygen and nitrite as electron acceptor (nitrite-DPAO). Thus, it can be concluded that biomass selected in both IFAS systems were mainly nitrite-DPAO.

Table 2-1 Phosphorus removal performance of IFAS systems with nitrate/nitrite shock load and variable electron acceptor; (1) varying mass of electron acceptor dosing during anoxic phase (2) Electron acceptor during anoxic phase was switched from nitrite to nitrate

	Test type	Electron acceptor	NO_x dosing	NO_x utilized	Anaerobic P release	Anoxic P uptake	Aerobic P uptake	Effluent
Units			mg N L^{-1}	mg N L^{-1}	mg P L^{-1}	mg P L^{-1}	mg P L^{-1}	mg P L^{-1}
IFAS-1	(1)	Nitrate	20	9	66.5	22	44.5	0.7
	(1)	Nitrate	40	10	65.3	21	44.3	0.7
	(1)	Nitrate	60	11	69.7	23.5	46.3	0.2
	(2)	Nitrite	20	20	69.6	24.9	44	1.3
	(2)	Nitrite	40	39	67.3	50.7	14.6	2.7
IFAS-2	(1)	Nitrite	20	20	75.9	29.3	46.5	0.2
	(1)	Nitrite	40	34	68.3	38.4	26.1	2.1
	(1)	Nitrite	60	30	69.8	41.5	25	3.3
	(2)	Nitrate	20	10	71.2	23	48.1	0.3
	(2)	Nitrate	40	13	69.6	18	51.8	0.2

Biomass in IFAS-2 was able to partially consume nitrate even though it was never exposed to nitrate. Martin et al. (2006) suggested that anoxic phosphorous uptake using nitrate as electron acceptor by biomass used to work only with nitrite is accomplished in two steps: first nitrate is reduced by bacteria (the so called flanking species present in the reactor) into nitrite and in the second step the produced nitrite is converted by nitrite-DPAO into nitrogen gas. When this hypothesis would be confirmed for a wider range of environmental conditions and carbon sources, this phenomenon should be carefully incorporated into activated sludge modelling and application to real wastewater.

The combined activity of flanking bacteria and nitrite-DPAO performing denitrification could also explain the partial utilization of nitrate observed during long term steady state and in different dosing kinetic tests in IFAS-1. The maximum specific anoxic phosphorus uptake rate in IFAS-1 (acclimated to nitrate) with 40 and 60 mg N L^{-1} of nitrite were 3.3 and 8.5 mg P h^{-1} g^{-1} $VSS_{(suspended + attached)}$, respectively, compared to 3.4 and 6.9 mg P $h^{-1}g^{-1}$ $VSS_{(suspended + attached)}$ in IFAS-2 where biomass were acclimated to nitrite. It confirms that the partial utilization of nitrate in IFAS-1 was not because of low number of DPAO; but, the selected DPAO could mainly perform denitrification from nitrite to nitrogen gas (nitrite-DPAO). The limiting step of denitrification in anoxic period in IFAS-1 was the first step by flanking bacteria as the most COD was up-taken by PAO/DPAO in the preceding anaerobic stage.

In IFAS-1 during long term steady state, phosphorous and nitrate were present simultaneously during anoxic condition. In spite of that, nitrate-DPAO could not develop to utilize the entire nitrate and enrich in the system. Guisasola et al. (2009) also reported that the nitrite-adapted DPAO could not switch to nitrate in a non-limiting phosphorous and nitrate condition over a long term operation. These findings show that denitrification ability of DPAO (nitrate-DPAO or

nitrite DPAO) is determined by factors other than only the type of electron acceptor provided during anoxic condition. Carvalho et al. (2007) found a correlation between denitrification ability of DPAO and the type of carbon source provided. DPAO in their reactors, fed with either acetate or propionate, were identified as nitrite-DPAO and nitrate-DPAO, respectively. It is in agreement with the results of this study as acetate was the sole volatile fatty acid provided and both IFAS systems with nitrate or nitrite could mainly select nitrite-DPAO. Identifying the other parameters affecting the denitrifying behaviour of DPAO (nitrate or nitrite-DPAO) is critical in order to determine DPAO contribution into biological nutrient removal process and updating the existing activated sludge models.

2.3.3 **Suspended and attached biomass characterisation**

Figure 2-4 exemplifies the results of a kinetic test (type 3 as per Methods) aimed at distinguishing the role of suspended and attached biomass of IFAS-1 and IFAS-2 separated into four batch reactors: R1 (suspended biomass of IFAS-1); R2 (attached biomass of IFAS-1); R3 (suspended biomass of IFAS-2) and R4 (attached biomass of IFAS-2).

Anaerobic phosphorus release and anoxic and aerobic phosphorous uptake were observed in all reactors. In R1 and R2 nitrate was partially utilized during anoxic stage (7 mg N L^{-1}) with respectively, 17 and 15 mg P L^{-1} anoxic uptake. In R3 and R4 nitrite was completely consumed and anoxic phosphorous uptake of respectively 18 and 17 mg P L^{-1} occurred. In R4, COD was not completely used up by PAO during the anaerobic stage and phosphorous release continued during the beginning of the anoxic period. During the aerobic period, simultaneous aerobic phosphorous uptake and nitritation (data not presented) occurred in all reactors. R1 and R3, containing suspended biomass, showed slightly higher ammonia removal and aerobic

phosphorus uptake than R2 and R4 containing attached biomass. None of the reactors could achieve complete phosphorous or ammonia removal.

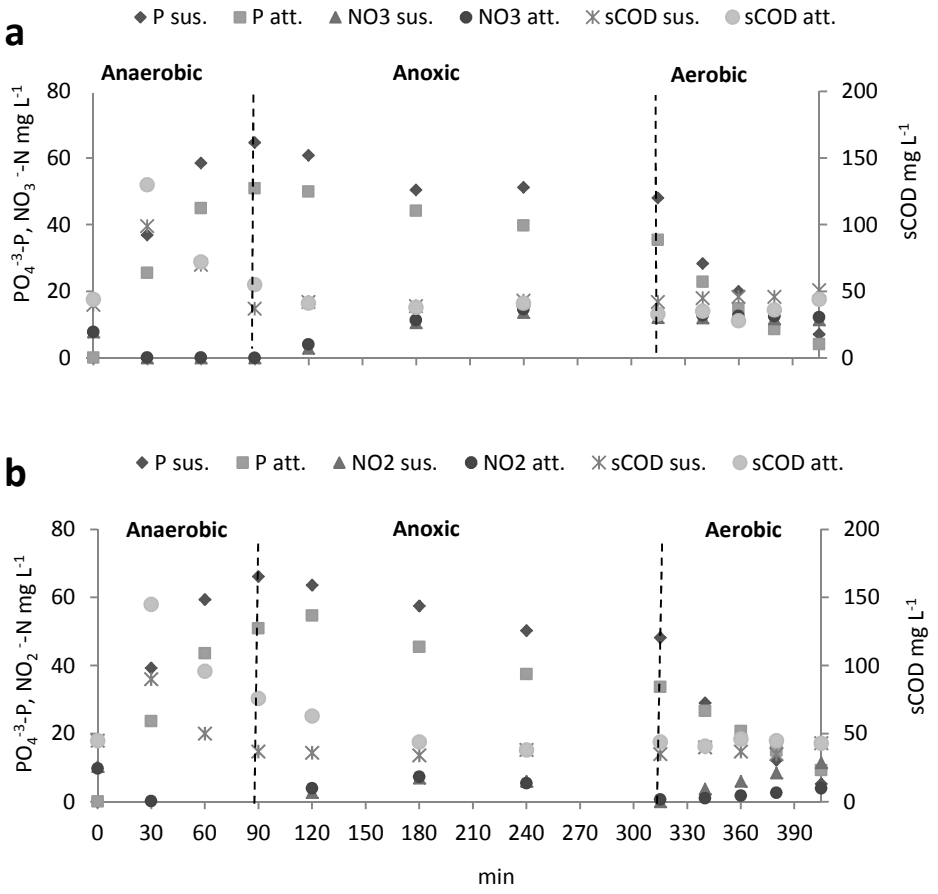

Figure 2-4 Concentration profile for phosphate, nitrite/nitrate and sCOD in: a) R1 (IFAS-1 suspended, nitrate) and R2 (IFAS-1 attached, nitrate) and b) R3 (IFAS-2 suspended, nitrite) and R4 (IFAS-2 attached, nitrite), (suspended biomass: sus.; attached biomass: att.)

Table 2-2 Specific biological reaction rates and nutrient removal contribution by suspended and attached biomass in this study (IFAS –1 and IFAS-2) and in the literature2 summarizes the results of these tests and compares them with literature. PAO/DPAO were present in both suspended and attached biomass of IFAS systems working with nitrate or nitrite as electron acceptor. The contributions of suspended and attached biomass to phosphorous removal were

similar between IFAS-1 and 2. This shows that the type of electron acceptor (nitrate/nitrite) below the inhibition or performance deterioration threshold did not affect the distribution of PAO in suspended and attached forms in IFAS systems.

Table 2-2 Specific biological reaction rates and nutrient removal contribution by suspended and attached biomass in this study (IFAS –1 and IFAS-2) and in the literature

	average $SRT_{suspended}$	Specific anaerobic P release rate (% of contribution)		Specific nitritation/nitrification rate (% of contribution)	
	d	mg P h^{-1} g^{-1} $VSS_{(suspended/attached)}$		mg N h^{-1} g^{-1} $VSS_{(suspended/attached)}$	
		suspended	attached	suspended	attached
IFAS-1 [a]	9	44.8 (56)	14 (44)	AOB: 5.7 (55)	AOB: 0.9 (45)
IFAS-2 [a]	9	46.8 (57)	14.1 (43)	AOB: 8.5 (68)	AOB: 1.4 (32)
(Onnis-Hayden et al., 2011) [b]	3.8	12.2 (97)	0.49 (3)	AOB+NOB : 2.5 (25)	AOB+NOB: 5.87 (75)
(Regmi et al., 2011)	4.8	-	-	AOB : 4.97, NOB: 1.72	AOB: 7.55, NOB: 0.82

In IFAS systems with biofilm carriers present only in the aerobic zone, the suspended biomass plays the main role in phosphorous removal (Kim et al., 2010; Onnis-Hayden et al., 2011). In this study in both IFAS systems, about 40% of phosphorus removal was carried out by attached biomass. Exposing attached biomass to alternating anaerobic/anoxic/aerobic conditions would select for PAO/DPAO in the attached form (Gieseke et al., 2002; Gonçalves and Rogalla, 2000; Helness and Ødegaard, 1999); however, the level of their contribution depended on availability of carbon during the anaerobic period. High COD concentration during anaerobic period (short filling time) means higher substrate penetration into the biofilm and in turn, poly hydroxyalkanoates storage by the attached PAO. Gieseke et al. (2002) reported that acetate could

fully penetrate the deep layers of biofilm at the feeding time (20 min) and COD loading comparable to this study. This suggested that the short feeding time in the IFAS reactors enabled attached bacteria to effectively compete with suspended biomass for substrate during the anaerobic period. This, combined with alternating anaerobic/anoxic/aerobic conditions, could explain the effective contribution of attached biomass into phosphorous removal in the IFAS systems.

Both IFAS systems maintained partial nitrification over long term of operation, with DO concentration maintained above 4 mg L^{-1} during the oxic period. Kinetic tests with only suspended or attached biomass also confirmed no nitrate accumulation in the systems. There are several factors, such as low DO, high pH, high temperature, FNA and free ammonia inhibition, short HRT and SRT that could lead to nitritation in activated sludge systems. The selection of AOB over NOB in suspended growth system is most likely achieved by short aerobic SRT of 1.7 d in the system (Blackburne et al., 2008; Munz et al., 2011). Attached biomass in the IFAS system is decoupled from the SRT. Thus, other factor/factors must have suppressed the growth of NOB in the attached biomass.

AOB selection over NOB in the attached biomass could be attributed to low DO condition in the biofilm. As oxygen penetrates the biofilm it is consumed by bacterial activity (aerobic phosphorus uptake and ammonia oxidation in this case) producing an oxygen gradient across the biofilm. Nitrifiers could not compete with PAO for oxygen and their activity is confined to sub-layers of the biofilm with low oxygen concentrations (Mosquera-Corral et al., 2005; Wu et al., 2009). The correlation between DO profile and spatial distribution of PAO, AOB and NOB in the biofilm were demonstrated in micro-scale studies (Gieseke et al., 2002; Okabe et al., 1999). The low DO condition in sub-layers of biofilm probably functioned selectively to maintain

nitritation as AOB have higher oxygen affinity compared with NOB (Liang et al., 2011; Ma et al., 2009). The oxygen-limited condition in the biofilm of both IFAS systems lasted almost entire oxic period as a sudden increase in DO was observed (Figure 2-2b and Figure 2-3b) near the end of the oxic period, coinciding with the completion of the phosphorus and ammonia removal.

The competition between the PAO and the nitrifiers for oxygen could cause a delay in the nitrification/nitritation process during oxic period (Gieseke et al., 2001; Gieseke et al., 2002; Zeng et al., 2011). In this study simultaneous nitritation and aerobic phosphorous uptake occurred in both suspended and attached biomass during aerobic period and full ammonia and phosphorous removal were achieved in a short aerobic time (1.5 h in 8 h cycle). Partial anoxic phosphorous uptake (40% of released phosphorus) and equal distribution of PAO between suspended and attached bacteria possibly lowered the oxygen uptake rate in each zone, allowing AOB to compete for oxygen and perform nitritation simultaneously with the aerobic phosphorus uptake.

Results highlighted the impact of PAO and nitrifiers' distribution on the IFAS system performance. The higher nitrification capacity in the IFAS system compared with activated sludge process was shown in a number of studies; however, only a few delineated the individual contribution of suspended and attached growth in the system. In a study by Regmi et al. (2011) full nitrification was achieved mainly by attached biomass (85%) at total SRT and the aerobic SRT of 4.8 d and 3.1 d, respectively. Onnis-Hayden et al. (2011) reported that 75% of full nitrification was carried out by biofilm in an IFAS system working at SRT of 3.8 d. On the other hand, van den Akker et al. (2010) found equal contribution of suspended and biofilm in an IFAS system at SRT of 7 to 9 d. A higher contribution of suspended biomass than biofilm to nitrification was observed at a full-scale IFAS plant operated at 8.5 d of SRT (Kim et al., 2011b).

In this study, IFAS systems at total SRT and aerobic SRT of respectively, 9 d and 1.7 d could only sustain partial nitrification and the suspended biomass showed relatively higher contribution compared with the attached biomass. Further studies are needed to determine the factors affecting the nitrifiers' distribution between the suspended and the attached biomass, in the presence of PAO activity.

2.4 Conclusions

In both IFAS systems full phosphorus and ammonia removal were achieved by PAO/DPAO and AOB activity. In both systems, regardless whether the biomass was acclimated to nitrite or nitrate, DPAO had preferred nitrite. PAO, DPAO and AOB were present in both suspended and attached forms and significantly contributed to phosphorous and ammonia removal. AOB could compete with PAO for oxygen in the biofilm; while, the DO-limited conditions in the biofilm suppressed NOB growth in the long term IFAS operation. The type of electron acceptor (nitrate or nitrite) did not affect PAO/DPAO distribution between the suspended and the attached biomass.

3 Chapter 3: Free nitrous acid inhibition of simultaneous denitritation and phosphorous removal in IFAS systems [2]

Abstract

Free nitrous acid (FNA) inhibition of anoxic and aerobic phosphorous removal in an integrated fixed-film activated sludge (IFAS) system with different FNA adaptation was investigated. A bench scale sequencing batch reactor (SBR) with plastic media was operated in an anaerobic/anoxic/aerobic sequence. During the anoxic period, nitrite was fed into the reactor at different concentrations to select for biomass adapted to 0.06 and 0.4 μg HNO_2-N L^{-1} of FNA during anoxic stage in Phase I and II, respectively. Long term anoxic/aerobic phosphorus removal was achieved in the IFAS reactor in both phases. In Phase I, aerobic phosphorous uptake was inhibited at higher level compared with anoxic phosphorus uptake. In Phase II, DPAO in both suspended and attached forms could adapt and were not inhibited at FNA level four times higher than the adapted concentration. The PAO's aerobic activity in Phase II did not show significant adaptation and was inhibited at slightly lower level compared with that in Phase I. The FNA inhibition of aerobic phosphorous uptake rate in attached biomass was 20% of that in suspended forms. In batch testes with the FNA level was raised to three times the adapted concentration, the contribution of attached biomass to overall anoxic and aerobic phosphorus uptake increased by 20% and 39%, respectively. The attached biomass could allow an IFAS

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system to be less inhibited and better maintain phosphorous removal at sudden FNA inhibition events.

3.1 Introduction

Integrated fixed film activated sludge (IFAS) system allows the co-existence of attached and suspended sludge in one bioreactor. This facilitates higher biomass concentration and diverse solids retention times (SRT) that provides higher volumetric treatment capacity (Ødegaard, 2006). The advantages of IFAS technology have been demonstrated widely for nitrogen removal from wastewater (Randall and Sen, 1996; Stricker et al., 2009). Recent pilot and full-scale tests have shown that IFAS systems could sustain enhanced biological phosphorous removal (EBPR). Sriwiriyarat and Randall (2005) reported that EBPR and nitrification in an IFAS reactor was achieved; however, its performance was affected by biomass distribution between media and suspended biomass and by the availability of carbon source in the anaerobic zone. In another study, Kim et al. (2011a) compared the nutrient removal performance of an IFAS system (media in the aerobic zone) with a conventional activated sludge process. In both systems, high COD and phosphorous removal were obtained at SRT of the suspended biomass ($SRT_{suspended}$) of 8 d. In all these studies phosphorous removal was carried out by suspended biomass of the systems.

The attached biomass in IFAS system could also contribute to biological phosphorous removal. Kodera et al. (2013) successfully used the attached biomass in a trickling filter to recover phosphorus as a concentrated phosphate solution from the effluent of a conventional activated sludge process. Phosphorous removal is conducted by selecting phosphorus accumulating organisms (PAO) through consecutive anaerobic and aerobic/anoxic conditions. For activated sludge processes with suspended biomass these alternative conditions could be achieved in different tank of zone; while, for attached biomass, could be introduced in to the system in time

sequences, as practiced in sequencing batch reactors (SBR) (Rogalla et al., 2006). The biological phosphorous removal has been mainly achieved through aerobic phosphorus uptake by PAO. A group of PAO, namely denitrifying PAO (DPAO), are able to uptake phosphorous using nitrite or nitrate as electron acceptor during anoxic condition (Oehmen et al., 2007). The anoxic phosphorous uptake by DPAO is of interest because it allows a reduction of carbon and aeration demand and sludge production. The DPAO's activity over nitrite in particular, bypasses the pathways of full nitrification and denitrification in conventional nitrogen removal, leading to considerable economic benefits (Peng and Zhu, 2006).

The presence of nitrite could affect both anoxic and aerobic phosphorous uptake. Studies showed that inhibition of anoxic/aerobic phosphorous uptake rate is correlated with the concentration of free nitrous acid (FNA), the protonated form of nitrite (Pijuan et al., 2010; Zhou et al., 2007). Thus the concentration of FNA should be considered when the phosphorous removal inhibition at the presence of nitrite is investigated. The FNA concentration (HNO_2-N) is estimated using Eq. 3.1 and 3.2 with T is temperature (℃) (Anthonisen et al., 1976).

$$FNA = \frac{NO_2^- - N}{K_a \times 10^{pH}} \qquad [3.1]$$

$$K_a = e^{-2300/(T+273)} \qquad [3.2]$$

Meinhold et al. (1999) observed that FNA at different concentration of 1.3 to 2.1 µg HNO_2-N L^{-1} (assuming tests were conducted at 20 ℃) inhibited the anoxic phosphorous uptake rate. They postulated that different FNA thresholds could be caused by the variable condition of the activated sludge used in the kinetic tests. In their experiment activated sludge was taken at different days from an anoxic/aerobic reactor of Biodenipho™ pilot plant treating real wastewater. Saito et al. (2004) used PAO/DPAO enriched sludge not adapted to FNA and found

complete inhibition of aerobic phosphorus uptake rate and 64% inhibition of anoxic phosphorous uptake rate at FNA concentration of 1.5 and 3 μg HNO_2-N L^{-1}, respectively. Sin et al. (2008) reported 75% and 37% inhibition of aerobic phosphorous uptake rate at 3 μg HNO_2-N L^{-1} of FNA in an activated sludge of a SBR and membrane bioreactor (MBR), respectively; the biomass in both reactors were intermittently exposed to nitrite, but the adapted FNA concentration was not determined. The studies by Yoshida et al. (2006) and Saito et al. (2008) showed that biomass adaptation should be considered when FNA inhibition of aerobic and anoxic phosphorous uptake activity are investigated.

So far, the inhibitory effect of FNA on aerobic and anoxic phosphorous removal have been studied mainly in suspended activated sludge systems. Zhou et al. (2007) reported that anoxic phosphorous uptake rate in granular activated sludge were inhibited at slightly lower level compared with flocculant biomass and proposed that attached biomass may have higher resistance to FNA inhibition compared with suspended biomass. In that study the granular and flocular biomass were selected in different bioreactors with different level of nitrite accumulation; while, the level of FNA adaptation was not reported. Wang et al. (2015) also observed a lower inhibition of DPAO's activity in granular activated sludge compared with flocculant biomass. In that study, DPAO were acclimated to nitrate as electron acceptor during anoxic condition. The fact that adaptation and other environmental factor could affect the FNA inhibition (Zhou et al., 2011) requires that experiments comparing FNA inhibition in suspended and attached biomass is conducted with biomass ideally selected in the same reactor with known FNA adaptation.

The objective of this study was to assess the FNA inhibition of anoxic and aerobic phosphorous removal in an IFAS system in which, both suspended and attached biomass performs biological

phosphorous removal. To characterize the effect of FNA adaptation, experiments were conducted in two phases with relatively low and high levels of FNA during anoxic conditions. Furthermore, the FNA inhibition of anoxic and aerobic phosphorous uptake in suspended and attached biomass were compared through kinetic tests using DPAO/PAO selected in the IFAS reactor with relatively high FNA adaptation.

3.2 Material and Methods

3.2.1 **Experimental set up**

IFAS system was set-up in a 3 L sequencing batch reactor (SBR). The SBR was seeded with sludge (both suspended and attached biomass) from a lab-scale IFAS system with alternating anaerobic/anoxic/aerobic cycle described by Jabari et al. (2014). The reactor was filled with media to 30% of the working volume. The media had a specific surface area of 500 m^2 m^{-3} (Anox Kaldnes, K1). Each cycle of operation consisted of 20 minutes filling period; 70 minutes anaerobic period, 225 min. anoxic and 90 min. aerobic period followed by 50 min. settling and 25 min. decanting period. HRT was 12 h and SRT of the suspended sludge was 10 d. Synthetic wastewater was used as feed with the following composition (all in mg L^{-1}): 240 CH_2COONa, 57.5 NH_4Cl, 51 K_2HPO_4, 83 $MgSO_4$, 13 $CaCl_2$, 65 yeast extract and 65 beef extract. The feed had 300 mg COD L^{-1}, 30 mg TN L^{-1} and 9 mg TP L^{-1}. During anaerobic and anoxic conditions nitrogen was purged in the reactor to prevent oxygen transfer from the air to the bulk. In aerobic condition, dissolved oxygen (DO) was controlled at 4.5 mg L^{-1}. The pH was controlled at 7.8±0.1 using 0.1 M HCl and NaOH solutions. Reactor was operated at room temperature (22±1℃).

The study was run for 5 months and IFAS reactor was operated in two phases. In Phase I during anoxic stage, total NO_2^--N of 60 mg (20 mg NO_2^--N per litre of the reactor) were dosed in eight

steps with an interval of 20 min. This manner of nitrite addition was performed to select for DPAO in low nitrite conditions. After one month steady state was achieved and reactor was operated for another month in this condition. In Phase II, total NO_2^--N of 105 mg (35 mg NO_2^--N per litre of the reactor) were dosed during anoxic stage in three steps to grow DPAO at elevated concentration of nitrite. Dosing was performed at minute 90, minute 150 with 15 mg NO_2^--N L^{-1} each time, and at minute 240 with 5 mg NO_2^--N L^{-1}. Steady state was achieved in three weeks and reactor was operated for two month under this condition.

3.2.2 **Kinetic study**

In both phases when steady state was achieved the inhibitory effect of FNA on DPAO and PAO were assessed through different kinetic tests. In Phase I nitrite of 20, 40 or 60 mg NO_2^--N L^{-1} was dosed to the reactor (in total of 60, 120 and 180 mg NO_2^--N, respectively). Dosing was conducted in one step (spike dosing) at the beginning of anoxic condition. In Phase II, suspended and attached biomass were separated using a 1mm pore size sieve. The size of activated sludge flocs are typically much less than 1 mm (Dahong and Ganczarczyk, 1991). After first screening, separated media were mixed with the reactor effluent to separate the potential suspended biomass attached to media. The screening was conducted again and the liquid was added to suspend biomass medium.The concentration of mixed liquor suspended solids (MLSS) in batch reactors containing media (attached biomass testes) in all cases was below 60 mg L^{-1}. The suspended biomass were settled and collected as well. The screening was conducted slowly to avoid detachment of attached biomass from media. Each biomass was then divided into four batch reactors. The new batch reactors were assessed for one cycle with an anaerobic (1.5 h)/anoxic (1.5 h)/aerobic (1.5 h) test. Four levels of nitrite (10, 20, 40 and 60 mg NO_2^--N/L) were injected (spike dosing) into the suspended and attached biomass reactors at the beginning of

anoxic condition. Same tests were conducted on the mixed suspended and attached biomass (IFAS). In all kinetic tests the feed was the same synthetic wastewater used for IFAS operation and pH was controlled at 7.8±0.1. Nitrogen was purged into the reactor during anaerobic and anoxic conditions.

In this manuscript, the maximum specific anoxic and aerobic phosphorous uptake rate (mg PO_4^{-3}-P $g^{-1}VSS_{S\&A}$; S: suspended, A: attached) are named aerobic and anoxic phosphorous uptake rate, respectively. The FNA concentration corresponding to each reaction rate was the average of FNA values over the time during which the rate was calculated.

3.2.3 **Analyses**

Nutrient removal performance of the reactor was monitored three times a week by measuring phosphate, ammonia, nitrate and nitrite at the beginning of the cycle and at the end of anaerobic, anoxic and aerobic stages. Mixed liquor concentration of the reactor was measured once a week. Monitoring nitrogen compounds and phosphorous during the cycle was carried out every two weeks. Phosphate, ammonium, nitrite and nitrate were measured by Lachat Instruments (Ontario, Canada) Quik Chem 8500, according to orthophosphate method 10-115-01-1-9 O, ammonia method 10-107-06-1-I and nitrate/nitrite 10-107-01-1-A, respectively. COD, mixed liquor suspended solids and mixed liquor volatile suspended solids (MLVSS) were measured according to Standard Methods (APHA, 2012). To measure the attached biomass concentration, ten biofilm carriers were randomly sampled and the attached biomass was scraped off and collected using pressurized water and a small brush. The produced liquid was filtered using 0.45 µm filter pore size and mass of filtrate and its volatile fraction was measured according to the method used for MLSS and MLVSS analysis, respectively. The total mass and the volatile solids (VS) to total

solids (TS) fraction of the biofilm in the reactors were then estimated using the total number of plastic carriers in the reactor.

3.3 Results and Discussions

3.3.1 **Nutrient removal performance in Phase I and II**

Excellent phosphorous removal was observed in the reactor in both phases and the phosphorous concentration in the effluent was below 0.2 mg PO_4^{-3}-P L^{-1} (Figure 3-1). The ratio of mg PO_4^{-3}-P released to mg of COD removed during anaerobic condition in phases I and II were respectively, 0.45±0.04 and 0.42±0.03. It shows that the ratio of PAO population to their competitor, glycogen accumulating organisms (GAO) in both phases were similar. The study by Pijuan et al. (2010) showed that presence of FNA during aerobic condition could favour GAO over PAO in biological phosphorous removal systems. In this study the increase in FNA concentration from 0.06 to 0.4 µg HNO_2-N L^{-1} during anoxic stage did not change the PAO/GAO ratio significantly. Anoxic phosphorous uptake was observed in both phases indicating the existence of DPAO. However, the anoxic uptake in Phase II was higher than in Phase I. In Phase I, the anoxic phosphorous uptake was approximately 30 mg PO_4^{-3}-P L^{-1} while in Phase II it increased to 45 mg PO_4^{-3}-P L^{-1}. Once the reactor operation was switched to Phase II, initially DPAO could not consume the additional nitrite. After three weeks of a transition period, DPAO gradually developed to utilize all the nitrite and anoxic phosphorous uptake increased. In both phases, the remaining phosphorous after anoxic period was fully removed during the aerobic condition.

In terms of nitrogen removal, in Phase I, during the aerobic period, ammonium was fully oxidized to nitrite and no nitrate production was observed. Partial nitrification, despite the DO of 4.5 mg L^{-1} during aerobic stage was explained by the short aerobic SRT of suspended biomass (1.7 days) and DO-limited conditions in the biofilm caused by PAO's activity in the IFAS

system. In Phase II, the concentration of ammonium in the effluent gradually increased and ammonia removal efficiency dropped to 10%. The low aerobic SRT for suspended biomass and competition with heterotrophs for oxygen in the biofilm suggest that the aerobic SRT in the IFAS reactor was close to the minimum SRT of AOB (Jabari et al., 2014). In such condition, any stress or operational change that reduces the growth rate of AOB marginally could make them gradually washed out of the reactor. The deterioration of nitritation process was coincident with the change in nitrite dosing at the beginning of Phase II. During the transient period, the nitrite accumulated during anoxic stage was transferred to the following aerobic condition; this combined with nitrite produced by nitritation resulted in the average FNA concentration of 0.52 µg HNO_2-N L^{-1}. This is significantly lower than the FNA concentration of 200 µg HNO_2-N L^{-1} reported to impose 50% inhibition on AOB activity (Hellinga et al., 1999); thus, perhaps other factors associated with high nitrite concentration could have disfavoured the AOB growth in the system. The knowledge of the changes in pH gradient and bacterial spatial distribution within biofilm during transient period may provide explanation to the cause of the AOB washout in Phase II.

The anoxic phosphorous uptake rate in Phase I and II were 4.7±0.8 and 8.0±0.9 mg PO_4^{-3}-P $g^{-1}VSS_{sus.\&att.}$ h^{-1}, respectively; the FNA concentrations during anoxic conditions were 0.06±0.01and 0.4±0.1 µg HNO_2-N L^{-1}. The higher FNA concentration in Phase II did not reduce DPAO's activity in the IFAS reactor in long term operation. Reversely, biomass could adapt to it and uptake phosphorous at higher rate because of higher concentration of electron acceptor. The aerobic phosphorous uptake rates were also comparable as 17.7±0.9 and 16.6±1.7 mg PO_4^{-3}-P $g^{-1}VSS_{sus.\&att.}$ h^{-1} in Phase I and II, respectively. In both phases complete utilization of nitrite during anoxic condition resulted in low FNA concentrations (<0.04 µg HNO_2-N L^{-1}) at the beginning of

aerobic stage; thus, the aerobic phosphorous uptake rate was not affected. In conclusion, the higher FNA concentration during anoxic stage in Phase II did not inhibit anoxic nor aerobic phosphorous removal performance of the IFAS system in long-term operation.

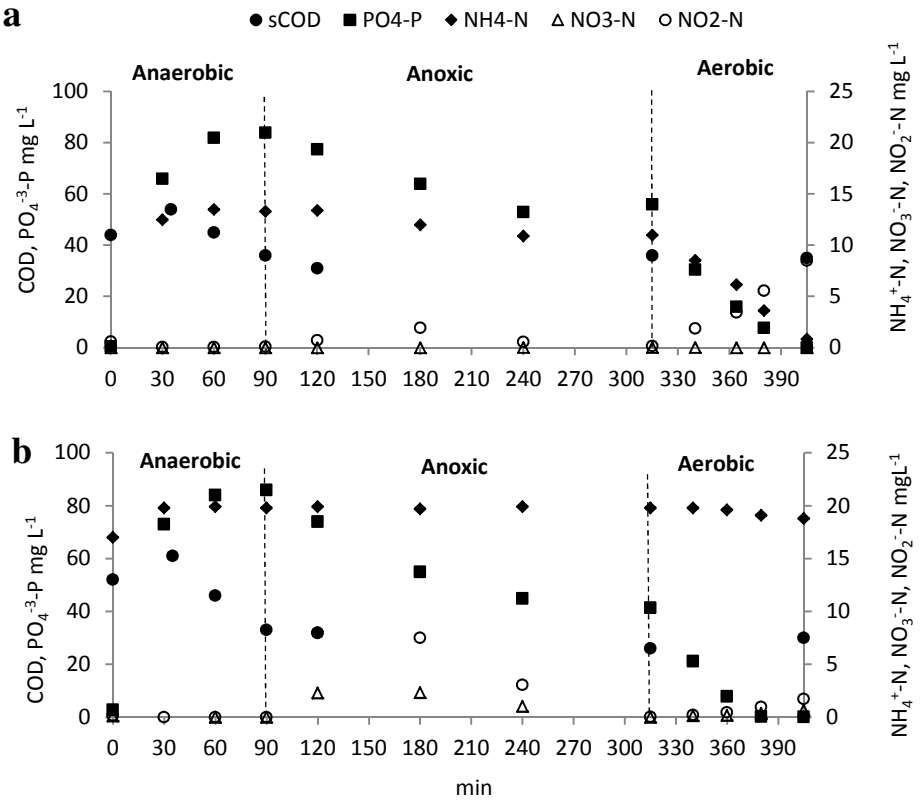

Figure 3-1 Cyclic profile of nitrogen, phosphorus, DO and FNA concentration in IFAS reactor during steady state in Phase I and II; sample at time zero was taken before feeding.

Average MLVSS and MLVSS/MLSS ratio in Phase I were 0.9 ± 0.1 g VSS L^{-1} and 0.73, respectively; these values in Phase II were 1.0 ± 0.1 g VSS L^{-1} and 0.77. The concentration of attached biomass in the volume of the reactor and its VS/TS ratio in Phase I were 2.0 ± 0.3 g L^{-1} and 0.81, respectively; these values for Phase II were 1.8 ± 0.2 g L^{-1} and 0.68. The FNA as an inhibitor could uncouple the energy and growth production and affect the biofilm formation (Schlag et al., 2007; Zhou et al., 2011). In the IFAS system, suspended and attached biomass are

exposed to different level of FNA, thus changes in FNA concentration in long term may affect the biomass distribution in suspended and attached forms. The study by Jiang et al. (2011) showed that FNA at concentration above 200 µg HNO_2-N L^{-1} in 6 to 24 hour could exhibit biocidal effect and reduce the viable fraction of anaerobic biofilm found in sewer systems. In this study, the change in FNA concentration between Phase I and II (0.4 µg HNO_2-N L^{-1}) was greatly lower and in long term it did not change the suspended and attached biomass concentration significantly.

3.3.2 **FNA inhibition on DPAO and PAO in the IFAS reactor in Phase I and II**

The FNA inhibition on DPAO and PAO in the IFAS reactor in Phase I was assessed with different nitrite dosing in batch tests (results shown in Table 3-1). The anoxic/aerobic phosphorous uptake rate vs different FNA concentrations in these tests and during steady state in Phase I are presented in Figure 3-2.

Table 3-1 Performance of IFAS reactor at different levels of nitrite dosing in phase I

Nitrite dosed	P release	Nitrite utilized	Anoxic P uptake	Aerobic P uptake
mg NO_2^--N L^{-1}	mg PO_4^{-3}-P L^{-1}	mg NO_2^--N L^{-1}	mg PO_4^{-3}-P L^{-1}	mg PO_4^{-3}-P L^{-1}
20	68.4	21.2	30.3	38.2
40	72.9	33.0	29.4	34.1
60	71.6	37.4	13.5	15.5

In the test with 20 mg NO_2^--N L^{-1} dosing, the same load of nitrite as regular operation was introduced in one spike into the reactor. The spike resulted in higher FNA concentration, however the anoxic phosphorous uptake rate was not inhibited and nitrite was completely utilized. The aerobic phosphorous uptake rate was also comparable with that in steady state and

phosphorous was removed completely. In the test with 40 and 60 mg NO_2^--N L^{-1} dosing, nitrite was partially removed and about 8 and 32 mg NO_2^--N L^{-1} were carried over to the following aerobic stage, respectively. In these tests, the high FNA concentration reduced the anoxic phosphorous uptake rate. The high FNA concentration during aerobic condition also reduced the aerobic phosphorous uptake rate and the inhibition increased with FNA concentration. The concentration of phosphorous at the end of aerobic stage was 9.7 and 47 mg PO_4^{-3}-P L^{-1} for 40 and 60 mg NO_2^--N L^{-1} dosing tests, respectively.

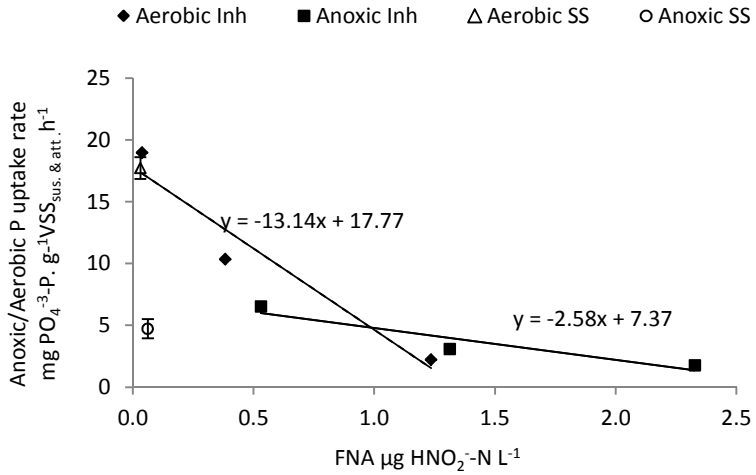

Figure 3-2 Anoxic/aerobic phosphorous uptake rate versus FNA concentration in steady state (SS)/inhibition tests (Inh) in Phase I

A line was fitted to the results in Figure 3-2 in order to compare the FNA inhibition of PAO' anoxic and aerobic activity. The aerobic phosphorous uptake rate was more inhibited than the anoxic phosphorous uptake rate, despite that the range of FNA concentration in anoxic stage was higher than that in aerobic condition. It is consistent with the results of Saito et al. (2004). On the other hand, Meinhold et al. (1999) and Zhou et al. (2012) reported that the anoxic phosphorous uptake was more sensitive to FNA inhibition than the aerobic phosphorous uptake. These

contradictory observations can be explained by how the concentration of nitrite affects the anoxic phosphorous uptake rate. Nitrite as the electron acceptor at higher concentration favours anoxic phosphorous uptake rate; while for nitrite as the precursor of the inhibition factor, FNA, a lower concentration is desired. Therefore, it could be expected that at low nitrite concentration the former effect is dominant and the anoxic phosphorus uptake rate would increase with nitrite concentration; after certain concentration the nitrite is not limiting the reaction rate anymore and the anoxic phosphorus uptake rate decreases because of the latter effect. In this study at the nitrite concentration of 20 mg NO_2^--N L^{-1}, the anoxic phosphorous uptake rate was slightly higher than that in regular IFAS operation (stepwise dosing of 20 mg NO_2^--N L^{-1} of nitrite), but higher nitrite concentrations produced lower anoxic phosphorous uptake rates. The nitrite concentration at which the inhibitory effect of nitrite becomes dominant depends on the microbial population, FNA adaptation and the suspended or attached type of the biomass.

In order to compare the inhibition of nitrite on aerobic and anoxic phosphorous uptake rates especially at low FNA concentration, the limiting effect of nitrite as the substrate for the anoxic phosphorous uptake rate should be excluded. Zhou et al. (2012) addressed this by conducting tests at high pH to create low FNA concentrations, so nitrite concentration did not limit the anoxic phosphorous uptake rate. They found that anoxic phosphorous uptake was inhibited at FNA concentration of 0.75 μg HNO_2-N L^{-1} and that anoxic phosphorus uptake was more sensitive to FNA inhibition than the aerobic phosphorous uptake. These results suggest that FNA could inhibit anoxic and aerobic phosphorous uptake rate at a wide range of concentrations and the level of inhibition depends on the biomass adaption to FNA and possibly other factors in the system.

In Phase II the performance of mixed (IFAS) or separated suspended and attached biomass were characterized at four different nitrite/FNA levels. Results of these tests are presented in**Error! Reference source not found.**. For all tests with 10 mg NO_2^--N L^{-1} and the IFAS test with 20 mg NO_2^--N L^{-1} dosing, nitrite was completely utilized during anoxic condition. In other tests, nitrite was partially consumed and the remaining nitrite was transferred to the aerobic stage. As a result, different levels of FNA were present at the beginning of aerobic stage and the aerobic phosphorous uptake rate decreased with the increase in FNA concentration.

Table 3-2 Performance of IFAS/suspended /attached biomass at different levels of nitrite dosing in Phase II

Test type	Nitrite dosed mg NO_2^--N L^{-1}	P release mg PO_4^{-3}-P L^{-1}	Nitrite utilized mg NO_2^--N L^{-1}	Anoxic P uptake mg PO_4^{-3}-P L^{-1}	Aerobic P uptake mg PO_4^{-3}-P L^{-1}
IFAS (suspended + attached)	10	68.8	10	10.5	50.5
	20	71.3	20	24.2	41.6
	40	64.6	23.3	24.5	27.1
	60	66.9	24.1	26.4	18.8
Suspended	10	74.6	10	7.0	40.9
	20	75.4	15.2	12.0	34.9
	40	75.8	15.6	12.0	22.0
	60	73.9	15.0	13.5	15.1
Attached	10	67.9	10	8.0	30.1
	20	67.4	17.2	14.0	29.2
	40	65.4	18.1	15.2	17.5
	60	67.0	20.0	16.0	16.8

The anoxic phosphorous uptake rates at different FNA concentrations in these tests are presented in Figure 3-3a. Results of IFAS tests showed that the anoxic phosphorous uptake was not inhibited with the increase of FNA concentration. This is different from Phase I (Figure 3-2)

where anoxic phosphorous uptake was inhibited at 40 and 60 mg NO_2^--N L^{-1} dosing tests. The reason is likely because the biomass in Phase II was adapted to the higher FNA concentration during anoxic stage. In the test with 60 mg NO_2^--N L^{-1} dosing, the anoxic FNA concentration is four times higher than FNA concentration during anoxic stage in Phase II (0.4±0.1 µg HNO_2-N L^{-1}), but the anoxic phosphorous uptake rate was not inhibited. This indicated that the FNA threshold could be higher than the adapted concentration. This could be because the long term operation at elevated FNA concentration induces development of certain metabolic pathways in bacteria or shift of microbial community to species with higher resistant to FNA inhibition which would remain unaffected at higher range of FNA. The long term effect of FNA on microbial community and enzymatic activity of the activated sludge system require further investigation.

The aerobic phosphorous uptake rates at different FNA concentrations are presented in Figure 3-3b. The aerobic phosphorus uptake rates at 10 and 20 mg NO_2^--N L^{-1} were comparable, but at higher nitrite dosing the rates significantly decreased. Comparing the slope of lines fitted to the aerobic phosphorous uptake rates for Phase I (Figure 3-2) and II (IFAS test in Figure 3-3b) indicated that the aerobic phosphorous uptake in Phase II was inhibited at slightly lower level compared with that in Phase I. This is in line with the results of Yoshida et al. (2006) and Saito et al. (2008); in their studies the biomass that was adapted to FNA during anoxic condition was inhibited during aerobic at relatively lower level compared to non-adapted biomass.

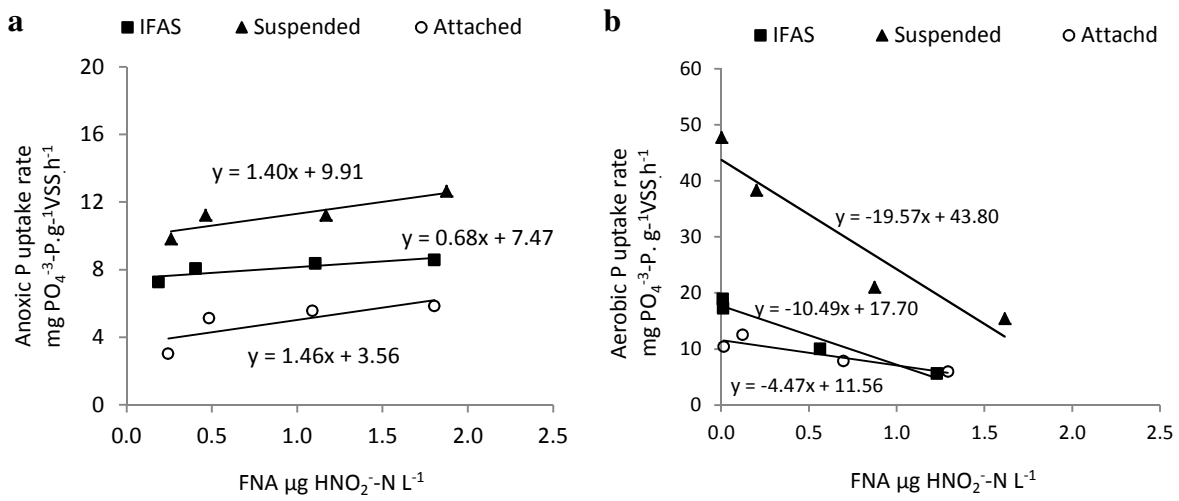

Figure 3-3 Anoxic /Aerobic phosphorous uptake rate of mixed (IFAS) and suspended or attached biomass in Phase II

The FNA inhibition on aerobic and anoxic phosphorous uptake in this study and literature are presented in Table 3-3 Comparison of FNA inhibition of biomass with different FNA adaptation and operational conditions. DPAO's activity of the biomass even with nitrate as electron acceptor (Saito et al., 2008; Yoshida et al., 2006) and presence of FNA during aerobic condition (Pijuan et al., 2010) both provide FNA adaptation for aerobic phosphorus uptake activity. Results of Phase II of this study also confirmed that FNA adaptation during anoxic condition could significantly increase the FNA inhibition threshold for DPAO's activity. However, comparing the results in some studies indicates that FNA adaptation solely could not explain the FNA inhibition and possibly other factors in the system also affect FNA inhibition of phosphorous removal. In the studies by Saito et al. (2004) and Yoshida et al. (2006) comparable FNA inhibition of aerobic phosphorus uptake was observed for adapted and non-adapted biomass. Pijuan et al. (2010) and Saito et al. (2008) reported complete inhibition of aerobic phosphorous uptake in non-adapted biomass at different FNA concentration of 9.8 and 1.4 µg HNO_2-N L^{-1}, respectively. The different type of wastewater, reactor and operational conditions used in these

studies could select for microbial population with different tolerance to FNA inhibition. Tora et al. (2010) reported that deficiency in inorganic nitrogen could enhance the FNA inhibition on AOB activity. More studies are needed to identify and characterize such factors effecting the FNA adaptation of PAO/DPAO biomass.

Table 3-3 Comparison of FNA inhibition of biomass with different FNA adaptation and operational conditions

Aerobic/Anoxic P uptake rate	Inhibition %	FNA tested µg HNO_2-N L^{-1}	Biomass FNA adaptation [c] µg HNO_2-N L^{-1} (A/Ax)[d]	Biomass type	Feed type	Reference
Aerobic	NR[a]	2.1 [b]	NR, DPAO activity	BiodeniphoTM pilot plant	NR	(Meinhold et al., 1999)
	100	1.5	NR, DPAO activity	PAO enriched in An/Ax (nitrate addition) /A SBR	synthetic	(Saito et al., 2004)
	92	1.2	Not adapted	An/A SBR	synthetic	(Yoshida et al., 2006)
	67	1.2	NR, DPAO activity [e]	An /Ax (nitrate addition) SBR	synthetic	
	51	1.2	NR, DPAO activity	An/A/Ax/A SBR	municipal	
	100	1.4	Not adapted	An/A (no nitrification) SBR	synthetic	(Saito et al., 2008)
	75	1.4	NR, DPAO activity	An/Ax (nitrate addition) SBR	synthetic	
	75	3	NR, periodic nitrite accumulation in aerobic stage	An/A/Ax/A SBR	synthetic	(Sin et al., 2008)
	37	3	NR, periodic nitrite accumulation in aerobic stage	MBR	synthetic	
	NR	0.05	0.02 (A)	An/A SBR (short cycle of 36 minute)	synthetic	(Freitas et al., 2009)
	90	9.8	0 - 1.9 (A-Ax)	An/A (partial nitrification)/Ax/2nd feeding/Ax SBR	abattoir	(Pijuan et al., 2010)
	100	9.8	Not adapted	An/A (no nitrification) SBR	synthetic	
	100	10	0.9 (A)	An/A/Ax/2nd feeding/An/A/Ax SBR	synthetic	(Zhou et al., 2012)
	88	1.2	0.06 (Ax)	An/Ax (nitrite addition)/A SBR	synthetic	This study Phase I
	72	1.2	0.4 (Ax)	An/Ax (nitrite addition)/A SBR	synthetic	This study Phase II
Anoxic	0	2.5	0.3 (A)	An/A/Ax(nitrate addition)/A SBR	synthetic	(Lee et al., 2001)
	36	3	NR, DPAO activity	An/Ax (nitrate addition) /A SBR	synthetic	(Saito et al., 2004)
	100	5	0.9 (A-Ax)	PAO enriched in An/A/Ax/2nd feeding/An/A/Ax SBR	synthetic	(Zhou et al., 2012)
	73	2.3	0.`06 (Ax)	An/Ax (nitrite addition)/A IFAS	synthetic	This study Phase I
	0	1.8	0.4 (Ax)	An/Ax (nitrite addition)/A IFAS	synthetic	This study Phase II

[a] NR: Not Reported, for the inhibition level, the inhibition incident was reported but not quantified and cannot be determined from the data presented.

[b] The value was estimated assuming the inhibition tests were conducted at 20℃.

[c] The FNA concentration that biomass was adapted to during aerobic (A) and/or anoxic (Ax) conditions. If adapted FNA concentration was not reported, it was estimated using pH, temperature and nitrite concentration of the sludge cultivation process found in the reference.

[d] A: aerobic, An: anaerobic, Ax: anoxic

[e] The relative DPAO activity measured as the ratio of anoxic P uptake to aerobic P uptake for this biomass was lower than that in other FNA adapted biomass in that study.

3.3.3 **FNA inhibition of suspended and attached biomass in Phase II**

The anoxic and aerobic phosphorous uptake of suspended and attached biomass tests (Table 3-2**Error! Reference source not found.**) indicated that DPAO and PAO were present in both suspended and attached forms; however, the FNA inhibition of their performance was different (Figure 3-3). For DPAO, the anoxic phosphorous uptake rate in both suspended and attached biomass was not inhibited when FNA concentration increased. This indicates that DPAO in both suspended and attached forms were able to adapt to FNA inhibition. In the case of attached biomass when nitrite dosing increased from 10 to 20 NO_2^--N L^{-1} the anoxic phosphorus uptake rate slightly increased. This is because nitrite at the concentration of 10 mg NO_2^--N L^{-1} limited the DPAO's activity as substrate in the biofilm.

The FNA inhibition on PAO's aerobic activity was observed on both suspended and attached biomass (Figure 3-3b). The aerobic phosphorus uptake rate decreased with the increased FNA concentration. Comparing the slope of lines fitted to the results indicates that aerobic phosphorous removal in suspended biomass was inhibited about five times higher than that in attached biomass. Different FNA inhibition on PAO's aerobic activity in biofilm and suspended biomass could be explained by different nitrite concentration and pH inside the biofilm and in bulk of reactor. As nitrite diffuses through the biofilm, its concentration drops and PAO are exposed to lower nitrite concentration in the biofilm (Gieseke et al., 2002; Wu et al., 2009). The pH gradient inside the biofilm affects the FNA-nitrite equilibrium (Park et al., 2010). Nanoparticle sensor used by Hidalgo et al. (2009) showed the existence of such pH gradient within the biofilm of a wastewater treatment process; however, the pH profile could vary for each system depending on the spatial distribution of species inside the biofilm (Bassin et al., 2012; Gieseke et al., 2002; De Kreuk et al., 2005). In this study lower nitrite concentration could

explain the lower FNA inhibition of PAO's aerobic activity in attached biomass. Since nitrification was lost in Phase II, nitrite in the bulk of the reactor was the only source of nitrite in the system and its concentration decreased with diffusion through the biofilm. This would result in lower FNA concentration inside the biofilm during aerobic conditions contributing to PAO being less inhibited.

At all levels of FNA, suspended biomass exhibited higher specific anoxic and aerobic rates than the attached biomass. Higher concentration of attached biomass in the reactor compensated for their lower reaction rate. The contribution of suspended and attached biomass to anoxic and aerobic phosphorous removal in the IFAS system at different FNA inhibition was estimated with E.3.3 with R is the anoxic/aerobic phosphorous uptake rate (mg PO_4^{-3}-P $g^{-1}VSS_{S/A}$ h^{-1}) and X is suspended /attached biomass concentration ($gVSS_{sus/att.}$ L^{-1}).

$$Contribution_{S/A}\ (\%) = \frac{R_{S/A}\,.\,X_{S/A}}{(R_S.X_S)+(R_A.X_A)} \times 100 \qquad [3.3]$$

Results are shown in Figure 3-4. The PAO/DPAO in the attached form could significantly contribute to anoxic and aerobic phosphorus uptake. The contribution of biofilm in the anoxic stage increased 19% with nitrite dosing increased from 10 to 20 mg NO_2^--N L^{-1}. That was because higher concentration of nitrite became available for DPAO in the biofilm. In aerobic conditions the contribution of attached biomass continuously increased up to 39% with nitrite dosing increasing from 10 to 60 mg NO_2^--N L^{-1}. These results suggest that at high level of FNA inhibition, PAO's activity in suspended biomass is significantly reduced, while PAO in biofilm are less inhibited. This could moderate the overall FNA inhibition of phosphorus removal performance in IFAS system.

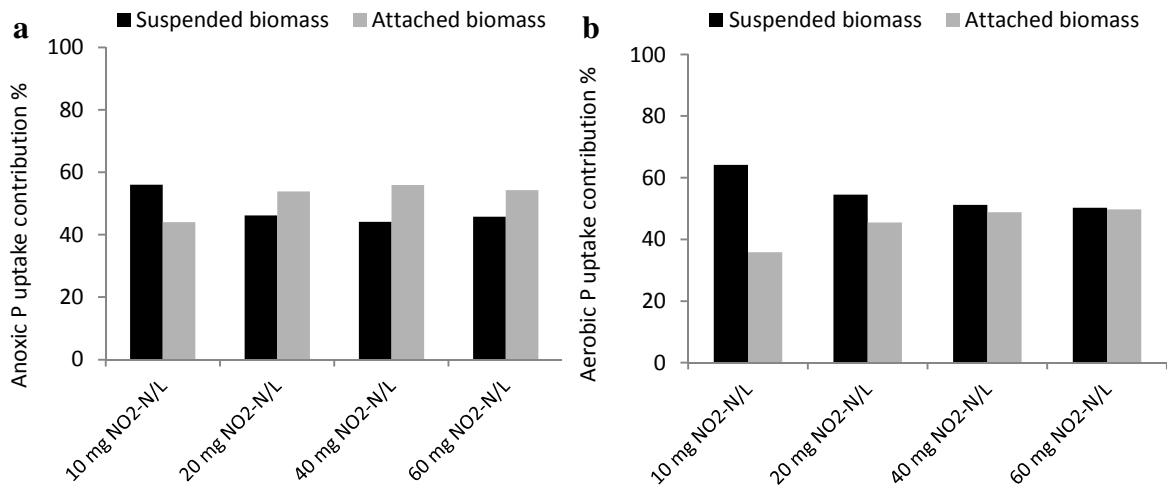

Figure 3-4 Contribution of suspended and attached biomass into a) anoxic and b) aerobic phosphorous uptake in IFAS system at different nitrite dosing tests in Phase II

3.3.4 **Effect of FNA on P/N ratio**

The ratio of phosphorous removed to nitrogen denitrified during anoxic conditions (P/N ratio) describes phosphorous removal efficiency of denitrification by biomass that would affect the nutrient removal performance of the treatment process. In activated sludge systems, DPAO compete with ordinary heterotroph organisms (OHO) and denitrifying glycogen accumulating organisms (DGAO) for electron acceptors during anoxic condition. The DGAO like DPAO could store carbon under anaerobic conditions, but utilize the stored energy during anoxic condition for denitrification to synthesize the glycogen, thus do not contribute to EBPR (Pijuan et al., 2011). The competition between DPAO, OHO and DGAO for electron acceptor during anoxic condition (Guisasola et al., 2009) and different FNA inhibition of denitrification and anoxic phosphorous uptake rates of the DPAO (Zhou et al., 2007) could cause different P/N ratio observed in different studies (Bassin et al., 2012; Coma et al., 2010; Meinhold et al., 1999; Saito et al., 2004). In this study in all tests, the rbCOD was completely depleted before anoxic stage (no further COD reduction during the anoxic and aerobic stage); thus the denitrification by OHO

was not significant and different effect of FNA on DPAO and DGAO performance was the probable cause for different P/N ratio (Pijuan et al., 2011).

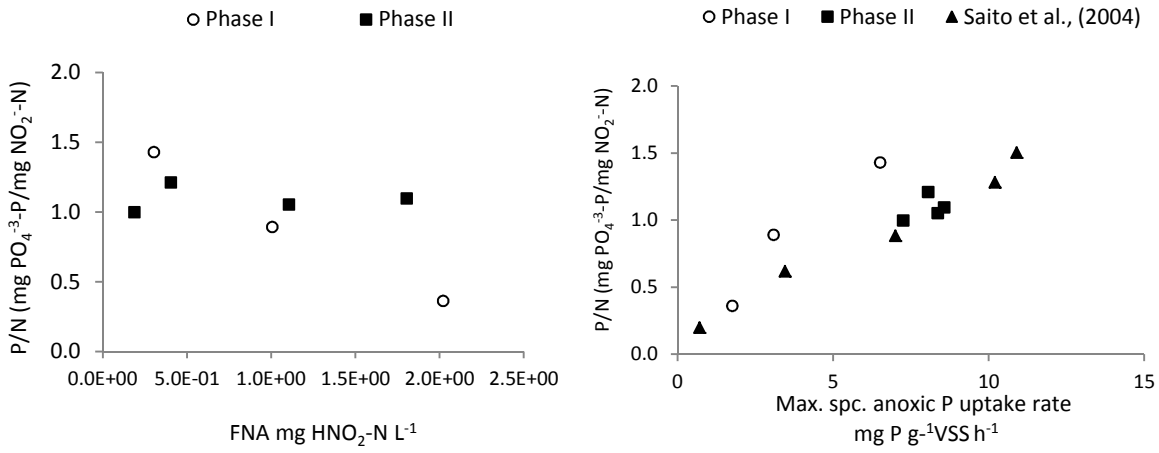

Figure 3-5 The P/N ratio versus a) FNA concentration and b) anoxic phosphorous uptake rate in inhibition tests in Phases I and II and tests conducted by Saito et al. (2004)

The P/N ratio versus FNA concentration calculated during the inhibition tests are presented in Figure 3-5a. In Phase I, the P/N ratio decreased with increased FNA concentration; while, in Phase II it was constant regardless of the increased FNA concentration. The experiments conducted by Ahn et al. (2001) and Saito et al. (2004) showed that FNA had negative impact on P/N ratio; in both studies the activated sludge was not acclimated to nitrite. In Phase I of this study the biomass was adapted to low FNA condition, the batch tests also showed that the P/N ratio was reduced when FNA level increased. In Phase II, where nitrite dosing strategy provided the biomass with sufficient FNA adaptation, the P/N ratio was not affected. Figure 3-5b showed that the P/N ratios are clearly correlated with anoxic phosphorus uptake rate. These results indicate that the P/N ratio was a function of anoxic phosphorous uptake rate and FNA adaptation could moderate the negative effect of FNA on P/N ratio.

In the activated sludge model ASM 2.d the anoxic phosphorous uptake is accounted for by using the kinetic equation for aerobic phosphorous uptake timed by anoxic reduction factor (Kuba et al., 1996b). It results in a constant P/N ratio for anoxic phosphorous uptake regardless of the FNA concentration and biomass FNA-adaptation (García-Usach et al., 2010; Sin et al., 2008). Results from this study and by Saito et al. (2004) shown in Figure 3-5b, suggest that P/N ratio could be found with an approximate linear function of anoxic phosphorous uptake rate. Further studies are needed to validate such an equation which could be used for dynamic model calibration for the treatment processes performing anoxic phosphorous uptake using nitrite as electron acceptor.

3.4 Conclusions

Long term anoxic/aerobic phosphorus removal was achieved in IFAS reactor with FNA concentration of 0.06 and 0.4 μg HNO_2-N L^{-1} during anoxic stage in Phase I and II, respectively. Higher FNA concentration in Phase II did not inhibit DPAO's activity. The DPAO in both suspended and attached forms could adapt and the IFAS reactor showed higher anoxic phosphorus uptake rate. The FNA inhibition threshold for both suspended and attached DPAO in Phase II was four times higher than the FNA-adapted concentration.

The DPAO/PAO in both suspended and attached forms significantly contributed to anoxic/aerobic phosphorus uptake. Under FNA inhibition in aerobic conditions, the PAO's activity in attached forms was five times less inhibited than that in suspended forms. In the batch tests with FNA level three times higher than adapted concentration, the contribution of attached biomass to overall anoxic and aerobic phosphorus uptake was increased by 20% and 39%, respectively. This indicates that the attached biomass at high FNA concentration could

effectively compensate for the suspended biomass inhibition and moderate the FNA inhibition of phosphorus removal in the IFAS system.

The phosphorus uptake to nitrite removed ratio (P/N ratio) was reduced by FNA at a function of anoxic phosphorous uptake rate. Adaptation to FNA was found to moderate the negative effect of FNA on P/N ratio. In Phase II, the ratio of phosphorous uptake to denitrified nitrogen at all FNA levels was constant at 1.1±0.1.

4 Chapter 4: Potential of hydrolysis of particulate COD in extended anaerobic conditions to enhance biological phosphorous removal [3]

Abstract

The effect of anaerobic hydrolysis of particulate COD (pCOD) on biological phosphorous removal in extended anaerobic condition was investigated through 1) sequencing batch reactors (SBRs) with anaerobic hydraulic retention time (HRT) of 0.8 h, 2 h and 4 h, 2) batch tests using biomass from a full scale biological nutrient removal (BNR) plant and, 3) activated sludge modelling (BioWin 4.1 simulation). The results from long term SBRs operation showed that phosphorus removal was correlated to the ratio of filtered COD (FCOD) to total phosphorus (TP) in the influent. Under conditions with low FCOD/TP ratio (average of 20) in the influent, extending anaerobic HRT to 4 h in the presence of pCOD did not significantly improve overall phosphorous removal. During the period with high FCOD/TP ratio (average of 37) in the influent, all SBRs removed phosphorous completely and the long anaerobic HRT did not have negative effect on the overall phosphorous removal. The batch tests also showed that pCOD at different concentration during 4 h test did not affect the rate of anaerobic phosphorus release. The rate of anaerobic hydrolysis of pCOD was significantly low and extending the anaerobic HRT was found to be ineffective. The simulation (BioWin 4.1) of SBRs with low influent FCOD/TP ratio showed that the default kinetics of anaerobic hydrolysis in ASM2d overestimated phosphorous removal in the SBRs (high anaerobic hydrolysis of pCOD). The

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default anaerobic hydrolysis rate in BioWin 4.1 (9 times lower than that inASM2d) could produce similar phosphorous removal to that in the experiment. Results showed that the current kinetics of anaerobic hydrolysis in ASM2d could lead to considerable error in predicting phosphorus removal in processes with extended anaerobic HRT.

4.1 Introduction

The efficiency of biological phosphorous removal in the wastewater treatment process depends on the amount of volatile fatty acids (VFA) that is available to phosphorus accumulating organism (PAO) during anaerobic condition (Merzouki et al., 2005; Mulkerrins et al., 2004). The VFA are a form of readily biodegradable carbon source (rbCOD) that could be present in the influent and also produced through fermentation of other forms of rbCOD. In many cases, the rbCOD in the influent is not sufficient for a complete phosphorous and nitrogen removal (Torrico et al., 2006). In these conditions, PAO are outcompeted by denitrifiers for available carbon and phosphorous removal deteriorates. A significant fraction of biodegradable COD in activated sludge systems are in the form of particulate COD (pCOD) which originates from the influent or is produced as a by-product of biomass decay (Morgenroth et al., 2002) . The pCOD consist of settleable and colloidal matter which first has to be hydrolysed to rbCOD through enzymatic and protozoan activity to become available to bacteria (Morgenroth et al., 2002).

The effect of pCOD on nutrient removal processes have been investigated by number of researchers. In studies conducted by Drewnowski et al. (2011; 2014) the settled wastewater was treated with coagulation-sedimentation and the biological nutrient removal (BNR) performance of activated sludge with original and treated wastewater was investigated. It was found that the pCOD removed (mainly colloidal matter) in such treatment had minor effect on anaerobic phosphorous release; but, the denitrification and anoxic phosphorous uptake rates were increased

by 46%. Torrico et al. (2006) studied the nutrient removal performance in a Dephanox-type reactor with raw and with settled wastewater. The anaerobic HRT of 4 h was provided in anaerobic tank and internal settler. The results showed that the nutrient removal with raw wastewater was higher. In a study by Puig et al. (2010) on a full scale BNR plant , 27% of raw wastewater by-passed the primary clarifier and directly entered to bioreactor. The authors reported that the operational change did not improve the nutrient removal performance. From their results it could be realized that during by-passing operation the influent had lower VFA to COD ratio compared with that during standard operation. Thus, the higher load of pCOD because of by-passing raw wastewater could have compensated for the lower VFA in the influent and as a result similar nutrient removal efficiency was achieved.

In another full scale study, two identical modified Bordenpho® process were operated in parallel with and without primary sedimentation (Tunçal et al., 2009). The latter showed higher phosphorous and TN removal and denitrifying PAO (DPAO) activity. It was reasoned that the pCOD provided additional carbon for denitrification during anoxic condition; consequently, the nitrate concentration in the return activated sludge was reduced and more rbCOD could be used by PAO (Tas et al., 2009). These studies indicated that pCOD in nutrient removal processes could improve phosphorous removal by providing additional carbon for denitrification and in turn less COD competition for PAO, but the direct effect of anaerobic hydrolysis of pCOD on PAO's activity could not be realized.

The effect of pCOD on biological phosphorous removal depends on the rate of hydrolysis process. The rate of hydrolysis process, particularly under anaerobic condition is lower compared with VFA assimilation and rbCOD fermentation (Morgenroth et al., 2002). In practice, the anaerobic HRT is usually designed for 0.25 to 1.5 h to allow for fermentation of available

rbCOD and assimilation of VFA (Barnard, 1984; Grady et al., 2011; Randall et al., 1998). In these cases, the effect of hydrolysis of pCOD might not be significant. However, typical variation in flow rate and composition of the influent could result that the biomass stays in anaerobic condition for extended time with no rbCOD available. In such conditions, the hydrolysis of pCOD could play an important role in phosphorous removal (Veldhuizen et al., 1999).

The rate of hydrolysis under anaerobic conditions is poorly investigated and current knowledge is contradictory. It is generally assumed that since the rate of anaerobic hydrolysis is relatively low; thus, to benefit from hydrolysis of pCOD the anaerobic condition should be extended (Grady et al., 2011). However, Wanger et al. (2015) reported that in an anaerobic/aerobic granular activated sludge-SBR system with rbCOD deficiency, extending anaerobic HRT form 1 h to 1.5 h did not improve phosphorous removal performance. With regard to activated sludge modeling (Henze et al., 2007), in ASM1, the rate of anaerobic hydrolysis is zero. In ASM3, rate of hydrolysis (only pCOD from influent) under all anaerobic/anoxic/aerobic conditions are equal. In ASM2d (commonly used for BNR modelling) it is assumed that rate of anaerobic hydrolysis is lower than that under aerobic condition and the rate of anaerobic hydrolysis is determined by applying a reduction factor to the rate of aerobic hydrolysis. The value of reduction factor varies significantly (0.1-1) in literature and it is usually determined in calibration to fit the simulation to experimental results (Drewnowski and Makinia, 2013; Sin and Vanrolleghem, 2006).

The objective of this study was to assess the effect of anaerobic hydrolysis of pCOD on phosphorous removal performance. The anaerobic hydrolysis of pCOD was mainly investigated by using kinetic tests with anaerobic condition of about 2 h (Choi and Lee, 2012; Drewnowski and Makinia, 2011; Drewnowski and Makinia, 2013; Goel et al., 1999; Puig et al., 2010). In this

study, the anaerobic HRT was extended to 4h to assure the potential effect of pCOD under anaerobic condition, even at low level, could be evaluated. The hydrolysis process was investigated in two ways, i.e. batch tests and bioreactor experiment. The long term bioreactor study enabled activated sludge community to acclimate to the operational conditions. This could resemble more reliably the anaerobic hydrolysis process in activated sludge systems. The experimental results were simulated in BioWin 4.1. Through this, the reduction factor that is used to determine the rate of anaerobic hydrolysis in ASM2d based models was estimated.

4.2 Methods

4.2.1 **SBRs operation**

Three sequencing batch reactors (SBR1, SBR2 and SBR3) with working volume of 3 L were set up and operated for two months. Reactors were seeded with BNR sludge form West End Water Pollution Control Center (WEWPCC), Winnipeg, Canada. The cycle of each SBR reactor consisted of: feeding (10 min), mixing period (0.8 h, 2 h and 4 h for SBR1, 2 and 3, respectively); aeration (3h); settling and decanting (1 h). The exchange volume ratio and SRT of reactors were 50% and 10 d, respectively. The HRT of SBR1, 2 and 3 were 9.6 h, 12 h and 16 h, respectively. The wastewater (primary influent) was delivered twice a week and stored at 4℃. Prior to feeding, the wastewater was transferred from fridge to a tank to bring the temperature to room conditions of 20±1℃ – the operating conditions for the reactors. The pH was not controlled and ranged from 7.2 to 7.9.

4.2.2 **Batch Tests**

To study the effect of hydrolysis of pCOD on anaerobic phosphorous release, wastewater with different concentrations of pCOD was used. First, primary influent was diluted with deionized

water to reduce the FCOD and in turn rbCOD concentration. Then, the diluted primary influent was filtered using ZeeWeed bench test unit (ZW-1, nominal porosity 0.02 μm) to prepare two different type of feeds: 1) concentrate feed with high content of settleable and suspended COD and, 2) permeate with only soluble COD (soluble feed). Separately, the original diluted influent was settled for 1.5 h (a typical retention time in primary clarifier) and certain amount of supernatant was discarded to produce a feed with both suspended and soluble COD (settled feed). The three different feeds prepared through physical separation would have similar rbCOD content but different pCOD content. Total of five batch reactors were set up. Each batch reactor was first supplied with 0.5 L of fresh BNR activated sludge from WEWPCC. Then, 0.5 L of the following prepared feeds: concentrate feed; settled feed; soluble feed; acetate solution (384 mg COD/L) and deionized water (control) were added to the reactors to make up a total volume of 1 L. The concentration of nitrate in the BNR sludge used for the batch tests was below 0.5 mg NO_3^--N L^{-1}. The batch tests were conducted for 4 h under anaerobic condition maintained by N_2 gas purging. The concentrations of MLSS, MLVSS, PO_4^{-3}-P and filtered COD were measured. These tests were repeated with the feeds prepared with 3, 4 and 5 times dilution of primary influent.

4.2.3 **BioWin simulation**

The BioWin simulation was conducted to study the effect of anaerobic HRT on phosphorous removal performance of the SBRs working in limited rbCOD conditions. BioWin 4.1 (Envirosim Inc., Ontario, Canada) uses an integrated model that is based on models ASM1, ASM2d and ASM3 by the International Water Association (IWA)(Henze, et al., 2007).

The simulation of SBRs were set up using the single-tank SBR unit in simulator and configured identical to SBR1 and 2 and 3 in the experiment. The settling times were set to be “reactive” in

terms of biological reactions to account for the denitrification observed during the settling time in SBRs. The temperature for all simulations was set 20℃. The SBRs were simulated for a period of 30 days of operation. Simulations were conducted using wastewater characteristic of the period day 53 to 64, to assess the SBRs' performance with low FCOD/TP ratio in the influent (limited rbCOD). The average concentration (mg L^{-1}) of total COD (TCOD) = 436, Filtered COD (FCOD) =135, total N (TN) = 57, total phosphorous (TP) = 6.9 of wastewater during this period were used for the simulation. The wastewater fractions were set using the measured influent characteristic and estimations through kinetic tests results; if data were not available, the default values were used. The details of wastewater characteristics and kinetic and stoichiometric parameters used in the calibrated model are presented in Appendix A.

In BioWin 4.1, similar to ASM2d, it is assumed that the rate of hydrolysis under anaerobic conditions is lower than that in the aerobic conditions. Therefore to calculate the rate of hydrolysis process under anaerobic condition, the equation used for aerobic hydrolysis is multiplied by a reduction factor named anaerobic hydrolysis reduction factor, η_{fe}. The default values for η_{fe} in BioWin 4.1 and ASM2d are 0.04 and 0.4, respectively. The value of η_{fe} in the simulation was varied to assess the effect of anaerobic hydrolysis of pCOD on phosphorous removal performance of simulated SBRs.

4.2.4 **Analysis**

During the SBRs operation, influent and mixed liquor before feeding and at the end of anaerobic and aerobic stages were sampled three times a week. The samples were analysed for TCOD, FCOD, TN, TP, NH_4^+-N, NO_3^--N, NO_2^--N and PO_4^{-3}-P. To measure the soluble parameters, samples were filtered through 0.45 µm filter. The concentration of pCOD was calculated as the

value of TCOD minus FCOD. The COD, TN, TP were measured using Hach test kits. The PO_4^{-3}-P, NH_4^+-N, NO_3^--N and NO_2^--N were measured by Lachat Instruments (Ontario, Canada) Quik Chem 8500, according to orthophosphate method 10-115-01-1-9 O, ammonia method 10-107-06-1-I and nitrate/nitrite 10-107-01-1-A, respectively. The concentration of MLSS and MLVSS were measured according to Standard Methods (APHA, 2012).

4.3 Results and discussion

4.3.1 **BNR performance of SBRs**

The characteristics of primary influent and SBRs performance are shown in Table 4-1 and Figure 4-1, respectively. In all reactors, full nitrification was observed during the entire experimental period (data not presented). The average concentration of nitrate in SBRs' effluent was similar (12.1 ± 4.8, 11.3 ± 4.5 and 10.2 ± 4.2 mg NO_3^--N L^{-1} in SBR1, 2 and 3, respectively), except for the period of day 51-66 when SBR1 showed lower nitrate removal efficiency than SBR2 and 3-discussed later with the results of SBRs' kinetic tests.

Table 4-1 Characteristics of primary influent

parameters	Value (STD) mg L^{-1}
TCOD	603 (±175)
F COD	220 (±85)
TN	65.1 (±9.7)
NH_4^+-N	28.5 (±4.5)
TP	9.0 (±2.4)
PO_4^{-3}-P	4.0 (±1.0)

The influent COD varied significantly and phosphorous removal in SBRs changed accordingly (Figure 4-1). The phosphorus removal performance of SBRs was closely correlated to the ratio

of FCOD/TP in the influent (Randall et al., 1998); for instance, during the periods of day 5-8, 17-22 and 32-45 with the high FCOD/TP of 41, 32 and 36, respectively, SBRs showed complete phosphorous removal. On the other hand, during periods of day 10-15, 29-31 and 51-64 with the relatively low FCOD/TP of respectively, 23, 16 and 23, the concentration of phosphorus in SBRs' effluent increased. The FCOD measured in this study accounts for the concentration of rbCOD, non-biodegradable soluble COD and partially colloidal COD with relatively low molecular size (Melcer, 2004; Wu et al., 2014). The concentration of non-biodegradable soluble COD in the influent was relatively constant because the concentration of COD in the effluent did not vary significantly (Tunçal et al., 2009) (FCOD of 48±8, 46±7 and 54± 12 mg L^{-1}in the effluent of SBR1, 2 and 3, respectively). Thus, the changes in FCOD concentration could approximately represent the variation of rbCOD (the required form of carbon for PAO) in the influent. This is confirmed by the correlation that was observed between the phosphorus removal efficiency of SBRs and concentration of FCOD in the influent.

During the experiment, the concentration of pCOD was always higher than FCOD in the influent; the average ratio of pCOD to TCOD in the influent was 0.63±0.07. During conditions with low FCOD/TP in the influent (limited rbCOD), the phosphorus removal in SBRs was similar. This indicated that pCOD in SBR2 and 3 during extended anaerobic HRT did not significantly provide additional carbon source for PAO to facilitate phosphorous removal performance. For example, on day 55 with influent FCOD/TP ratio of 23 and pCOD and FCOD concentrations of 257 and 156 mg L^{-1}, respectively, all SBRs had similar concentration of phosphorus in the effluent (3.7 mg PO_4^{-3}-P L^{-1}). This indicated that the availability of pCOD for phosphorus removal under anaerobic condition was limited in the activated sludge system.

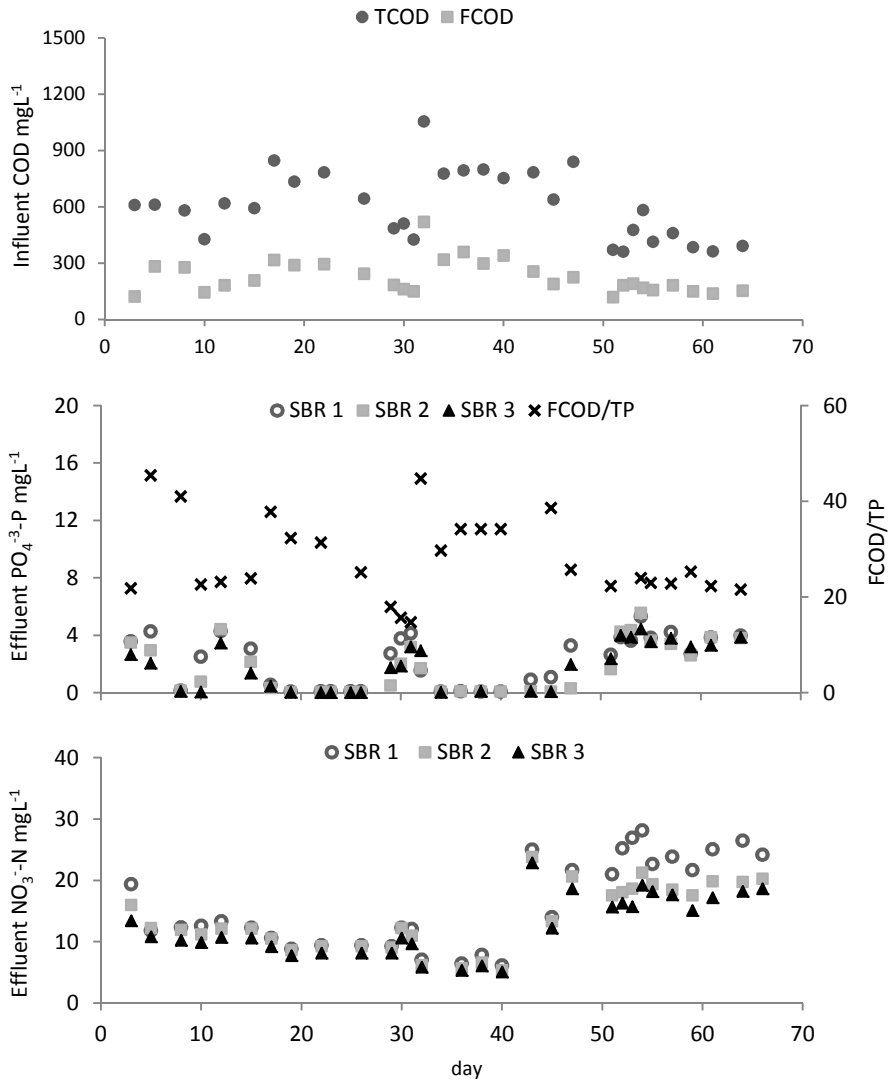


Figure 4-1 Historical profile of influent COD and effluent phosphorous

The cyclic profile of phosphorous, nitrogen compounds and COD in SBRs on day 38 (influent FCOD/TP = 34) and 54 (influent FCOD/TP = 24) are presented in Figure 4-2. On day 38, all reactors were able to remove phosphorous completely and nitrate concentrations in the SBRs' effluent were similar. The maximum specific anaerobic phosphorous release rates for SBR1, 2 and 3 were 7.4, 6.4, 5.3 mg PO_4^{-3}-P $g^{-1}VSS^{-1}$ h^{-1} respectively, and accordingly, the maximum specific aerobic phosphorous uptake rates were 3.8, 5.8, 6.5 mg PO_4^{-3}-P $g^{-1}VSS^{-1}$ h^{-1}. The results

showed that there is a correlation between the anaerobic HRT and the rate of anaerobic phosphorous release and aerobic phosphorous uptake. With the increase of anaerobic HRT, the rate of anaerobic phosphorous release decreased; while, the rate of aerobic phosphorous uptake increased. Similar observation were reported by Coats et al. (Coats et al., 2011) who studied SBRs with anaerobic HRT of 1, 2 and 3 h. In order to fully understand the impact of anaerobic HRT on the phosphorous release and uptake, the poly-phosphate (Poly-P), poly hydroxy alkanoates (PHA) and glycogen transformation in reactor operated with different anaerobic HRT should be investigated.

The kinetic study of SBR3 on day 38 showed that (Figure 4-2), phosphorus was released at relatively high rate in the first 60 min (simultaneous with high rate FCOD reduction because of rbCOD uptake); afterwards, its rate was reduced. It is assumed that PAO in the absence of electron acceptor continuously use poly-P for maintenance energy (Lu et al., 2007)**.** Since phosphorous released in this way is not used for PHA storage, it is reported that long anaerobic HRT could reduce the net phosphorous removal and upset the phosphorous removal efficiency (Barnard, 1984). In this study, 4 h of anaerobic HRT in SBR 3 did not have negative impact on the phosphorous removal performance. Similarly, Coats et al. (2011) observed that extending anaerobic HRT up to 3 h in an anaerobic/aerobic SBR did not cause deterioration of phosphorous removal. It was reported that phosphorous released in the sludge layers of a secondary clarifier with relatively high retention time of 3 to 4.5 h was reabsorbed when oxygen or nitrate was provided and the phosphorous concentration in the effluent was not affected (Mikola et al., 2009; Wouters-Wasiak et al., 1996). These findings suggest that extending anaerobic HRT to some extent (4 h of anaerobic HRT in this study) is not detrimental to phosphorous removal efficiency.

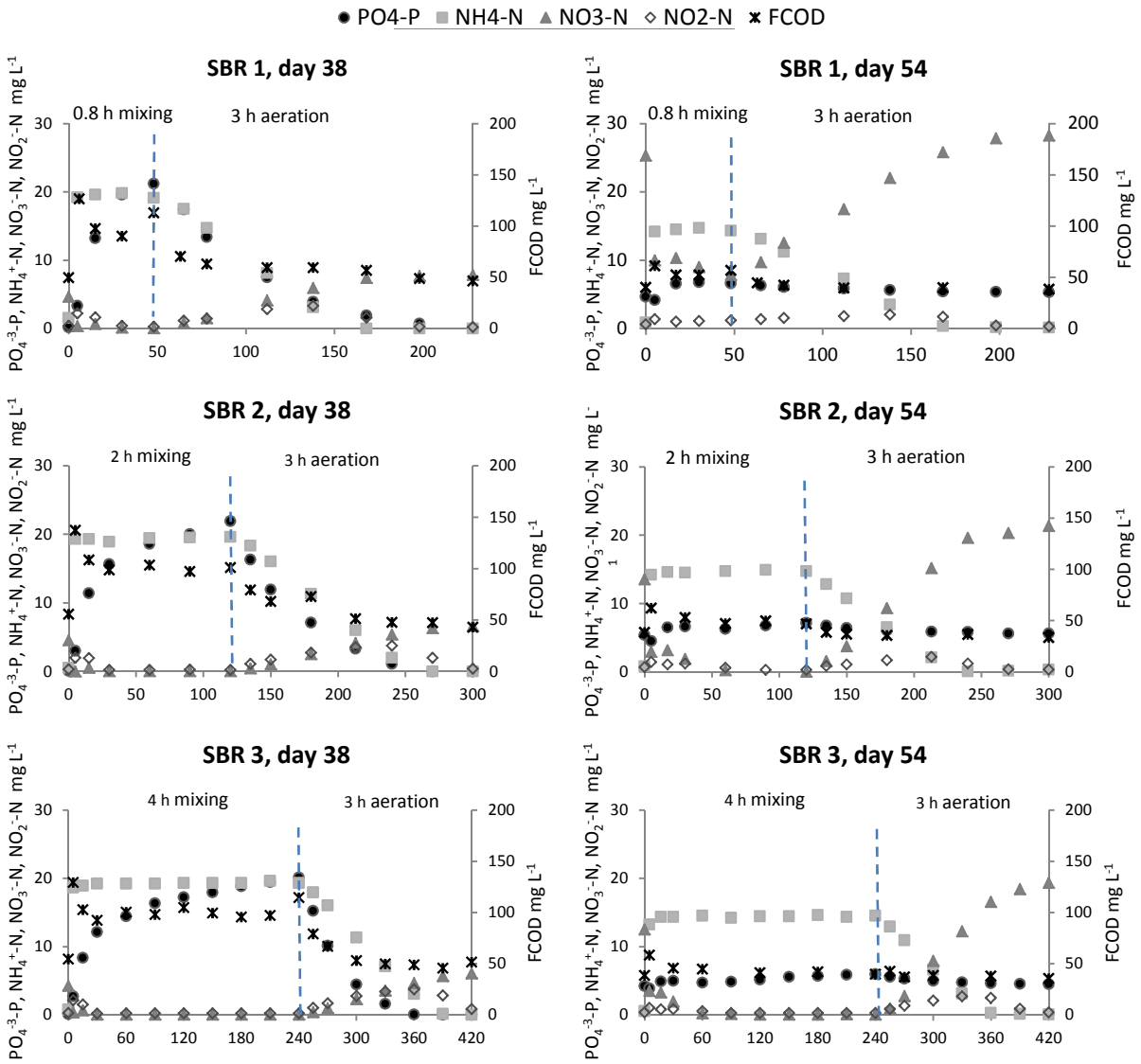


Figure 4-2 Cyclic profile of FCOD, phosphorous and nitrogen compounds in SBRs at day 34 (low FCOD/TP in the influent) and day 54 (high FCOD/TP in influent)

The kinetic study on day 54 (low FCOD/TP in the influent) showed that none of the reactors had PAO's activity (negligible phosphorus release during anaerobic conditions). This suggested that the long anaerobic HRT at the presence of pCOD (influent pCOD= 413 mg L^{-1}, influent FCOD=168 mg L^{-1}) in SBR3 did not benefit PAO's activity. On the other hand, SBR2 and 3 had lower nitrate in the effluent than SBR1. The anoxic/anaerobic period in SBR2 and SBR3 were

long enough that nitrate recycled from previous cycle was removed completely. In SBR1, 0.8 h of mixing period (anoxic) was not sufficient for complete denitrification and nitrite was transferred to the following aerobic stage. As a result, the concentration of nitrate in the effluent of SBR2 and 3 was lower than that in SBR1. In SBR2 and 3, the nitrate was removed in one hour of beginning of mixing period and the extended anaerobic condition at the presence of pCOD could not provide additional carbon for PAO.

4.3.2 **Anaerobic hydrolysis of pCOD in batch tests**

Total of 3 sets of batch tests with different dilution of primary influent were conducted. The results of these three sets were consistent. For simplicity, only one set of the results are presented (Figure 4-3). In this test, the feeds were prepared with 3 times dilution of primary influent. The concentrations of COD in the feeds used in this set of test are shown in Table 4-2.

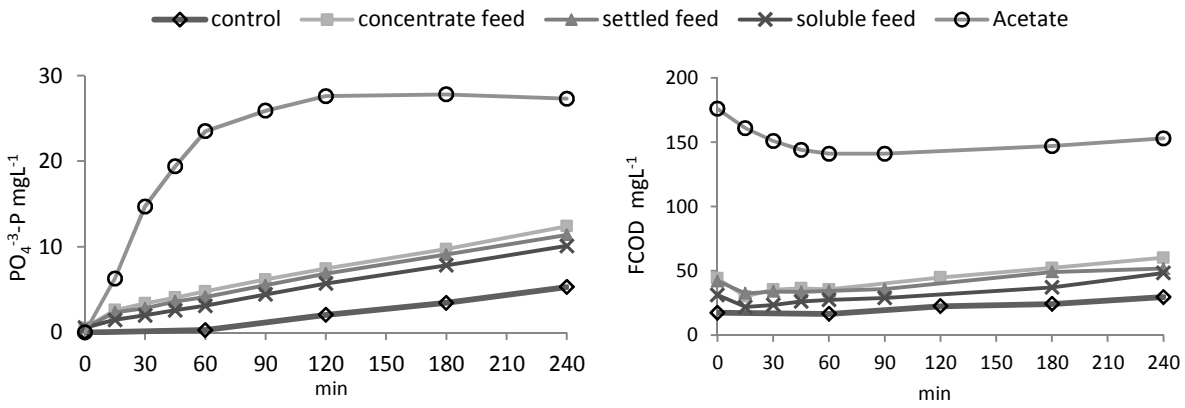


Figure 4-3 Concentrations of phosphorous and FCOD in anaerobic batch tests

In the test with acetate in the feed, phosphorous was released to its maximum level within the first 90 min of the anaerobic condition. This indicated that the BNR activated sludge contained PAO with poly-P storage. Therefore, the VFA that became available to PAO in any batch test through feed or caused by hydrolysis of pCOD would be realized through the rate of

phosphorous release in that test. In the control reactor, the phosphorus release was delayed for 60 min. It could be that oxygen was partially present in deionized water used as feed. In the tests with concentrate, settled and soluble feed, phosphorus was released at similar rates of 3.0, 2.8 and 2.7 mg PO_4^{-3}-P $g^{-1}VSS^{-1}$ h^{-1}, respectively. It was noted that in first 15 min of these tests, phosphorous release rate was slightly higher than that in the rest of anaerobic time. It is probably because small concentration of rbCOD existed in the prepared feeds (a slight reduction of FCOD occurred during this time).

Table 4-2 Concentration of pCOD and FCOD in feeds used for batch tests

	concentrate feed	settled feed	soluble feed	VFA
COD type	settleable + suspended+ soluble	suspended + soluble	soluble	Acetate
pCOD [a] (mg L^{-1})	181	69	0	0
FCOD (mg L^{-1})	55	49	38	384

[a] The pCOD concentration was calculated as the measured TCOD minus the measured FCOD

In the tests with concentrate, settled and soluble feeds, any available rbCOD in the feeds would be depleted immediately (high rate phosphorous release and FCOD reduction in first 15 min); afterwards, the phosphorous release could be because of different reasons. Besides the pCOD present in the concentrate and settled feeds, the activated sludge itself contained pCOD that was mainly derived from decay of biomass (Morgenroth et al., 2002). The pCOD could be hydrolysed during anaerobic condition and provides PAO with rbCOD to facilitate phosphorus release. The decay of PAO also could result in phosphorous release (Hao et al., 2010a). The phosphorous release associated with activated sludge was equal in all reactors as they had the same type and concentration of activated sludge. The higher concentration of pCOD in tests with

concentrate and settled feeds did not increase the rate of phosphorous release during 4 h anaerobic conditions. This showed that the availability of pCOD to PAO was limited by low rate of hydrolysis process under anaerobic conditions. This is in line with the results of SBRs experiment.

In this study the anaerobic hydrolysis of pCOD present in a municipal wastewater was assessed with two different types of activated sludge (lab-scale anaerobic/aerobic SBR and full scale BNR system). In both cases, the anaerobic hydrolysis of pCOD could not provide significant carbon source for PAO's activity in 4 h of anaerobic condition. Mąkinia et al. (2011) also reported that the effect of pCOD on anaerobic phosphorous release of activated sludge from two different full scale plants was not significant. Therefore, the very low rate of anaerobic hydrolysis found in these studies might not be due to the specific activated sludge and wastewater tested. This suggested that the rate of hydrolysis of pCOD was generally negligible under anaerobic conditions. On the other hand, Goel et al. (1999) reported that the anaerobic conditions did not affect the extracellular enzymatic activities associated with the hydrolysis process; thus, the rate of hydrolysis should be comparable at different redox conditions. This contradiction could be because other extracellular processes such as protozoan activity that play an important role in hydrolysis of pCOD are less active in anaerobic condition (Dubber and Gray, 2011; de Kreuk et al., 2010; Morgenroth et al., 2002)

4.3.3 **Results of BioWin 4.1 simulation**

The effect of anaerobic hydrolysis of pCOD could be assessed by comparing the phosphorous removal performance of anaerobic/aerobic SBRs with different anaerobic HRT under low bCOD condition. The average concentration of phosphorous and nitrate in SBRs' effluent during day 53-64 with low FCOD/TP in the influent (limited rbCOD) and the results of simulation for this

period are compared in Figure 4-4. The concentration of effluent ammonium and nitrite in all simulations were negligible. The simulation was conducted at different anaerobic hydrolysis rates by changing the anaerobic hydrolysis reduction factor, η_{fe}.

The simulation with default value of η_{fe} in BioWin 4.1 (0.04) predicted slight difference in phosphorous removal among SBRs; the nitrate concentration in the effluent of SBR1 was relatively higher than that in SBR2 and 3; these were consistent with experimental results. The low rate of anaerobic hydrolysis produced by η_{fe} of 0.04 did not lead to high VFA production during anaerobic time and consequently phosphorous removal in SBR2 and 3 was not improved significantly. On the other hand, when η_{fe} was set 0.4 (default in ASM2d), the phosphorous removal significantly improved with increase in anaerobic HRT. The nitrate concentration in the effluent was not affected with different values of η_{fe}. It was because the hydrolysis rate under anoxic conditions in BioWin 4.1 simulator similar to ASM2d is determined separately by the anoxic hydrolysis reduction factor that was constant in all simulations.

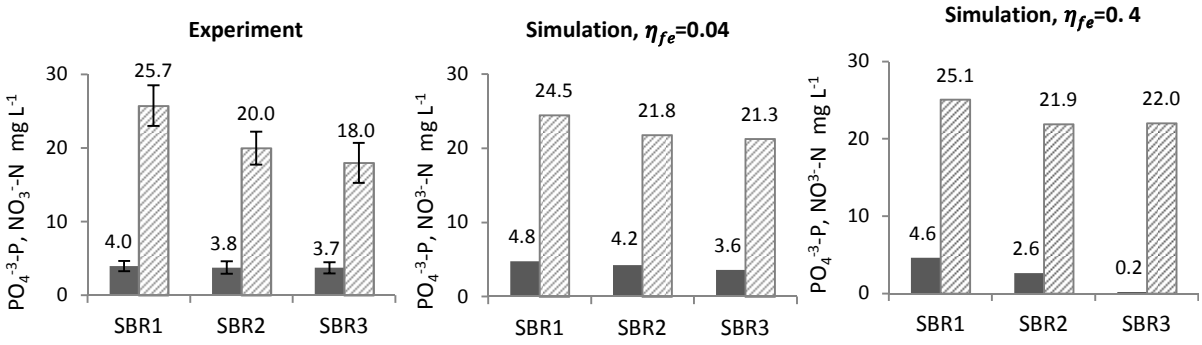


Figure 4-4 Nutrient removal of SBRs during influent low FCOD/TP and its simulation results

In activated sludge modelling since the rate of anaerobic hydrolysis is unknown, η_{fe} is usually selected to fit the simulation results to the experimental values. As a result, a wide range of values for η_{fe}have been used in different researches. Sin et al. (2006) used different values of 0.4

and 1 for η_{fe} for calibration of the ASM2d model to simulate an anaerobic/aerobic SBR at different operational configuration. The calibrated model still could not satisfactorily predict the dynamics of phosphorous removal and hydrolysis process. Mąkinia et al. (2006) used η_{fe} of 0.1 to calibrate the model of a full scale nutrient removal system. The anaerobic hydrolysis rates produced by these values would result in different rbCOD production that could falsely affect the result of simulation for phosphorous removal at low rbCOD condition. To illustrate that, the SBR2 and 3 were simulated with the value of η_{fe} varied from 0 to 1. The difference of phosphorous concentrations in the effluent of SBR2 ($HRT_{anaerobic}$= 2h) and SBR3 ($HRT_{anaerobic}$= 4h) were used at each η_{fe} to calculate the phosphorous removal associated to hydrolysis of pCOD per hour of anaerobic condition. Results of these simulations are presented in Figure 4-5.

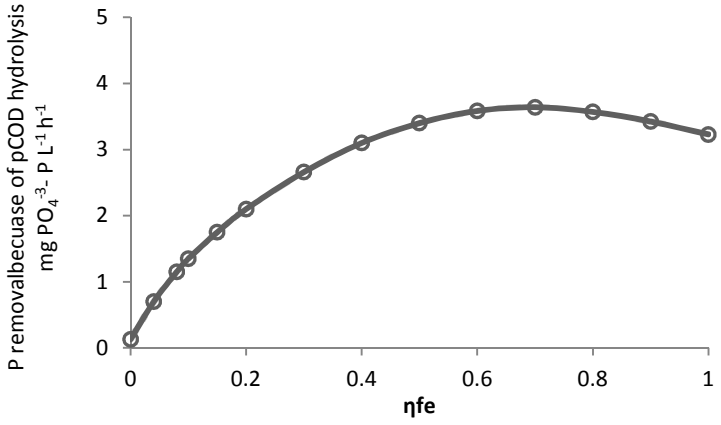


Figure 4-5 Effect of pCOD at different η_{fe} on phosphorous removal in simulation

The phosphorous removal associated with anaerobic hydrolysis of pCOD increased with the value of η_{fe} as more pCOD would be hydrolysed. At values above 0.5 for η_{fe}, the beneficial effect of pCOD on phosphorous removal decreased. It was because in SBR3, the relatively high rate of hydrolysis during 4 h of anaerobic HRT significantly reduced the pCOD content in the activated sludge that limited the rbCOD production.

With the default value of η_{fe} in ASM2d (0.4), the beneficial effect of anaerobic condition would be 3 mg PO_4^{-3}-P $L^{-1}h^{-1}$ that is significant in a phosphorous removal system. The estimated effect of pCOD hydrolysis on phosphorous removal depends on other values used in the model, in particular the stoichiometric parameters for PAO. Nevertheless, these results suggest that in calibration of activated sludge model with regard to the value of η_{fe}, it has to be checked if the model predicts realistic phosphorous removal at limited rbCOD condition. In this study a relatively low value of η_{fe} could provide realistic prediction for phosphorous removal performance when the concentration of rbCOD in the influent was not sufficient for PAO's activity.

Simulation results showed that the anaerobic hydrolysis of pCOD, depending on the value of reduction factor, could significantly affect the results of phosphorous removal. The hydrolysis process is mainly investigated in aerobic conditions. The methods developed to estimate the kinetic parameters of hydrolysis process and model modifications are mainly based on aerobic metabolism of activated sludge, e.g. oxygen uptake rate (Eliosov and Argaman, 1995; Insel et al., 2002; Insel et al., 2003; Marani et al., 2004; Orhon et al., 1998; Tas et al., 2009). The rate of anaerobic hydrolysis is produced by applying a reduction factor to the kinetics estimated for aerobic hydrolysis. Studies have shown that the hydrolysis mechanisms could be different under aerobic and anaerobic conditions (e.g. different protozoan activity) (de Kreuk et al., 2010; Morgenroth et al., 2002); thus, model modifications and kinetics estimated based on results from aerobic respiration might not be applicable for anaerobic hydrolysis process. For example, Orhon et al. (1998) modified the model of aerobic hydrolysis in ASM2d by incorporating a new variable of "readily hydrolysable pCOD". They found that the modified model could predict more accurately the oxygen uptake rate (OUR) of activated sludge with both municipal and

industrial wastewater. Drewnowski et al. (2013) compared the proposed model with ASM2d for modelling a BNR activated sludge system and found the modified model, except for OUR results, could not improve the accuracy of results for anaerobic phosphorous release and anoxic/aerobic phosphorous uptake rates. Therefore, there is a critical need to further the understanding of the mechanism of hydrolysis process under anaerobic condition and to develop specific model for its kinetic estimation.

4.4 Conclusions

The effect of anaerobic hydrolysis of particulate COD (pCOD) on biological phosphorous was investigated by comparing phosphorous removal performance of three SBRs with different anaerobic HRT. It was found that the phosphorus removal efficiency of SBRs was closely correlated to the ratio of FCOD/TP of the influent. With low FCOD/TP ratio (average of 20) in the influent, extending anaerobic conditions to 4 h in presence of pCOD did not benefit the PAO's activity and all SBRs showed similar low level of phosphorous removal. When the ratio of FCOD/TP increased in the influent (average of 37), all SBRs achieved complete phosphorus removal and long anaerobic conditions did not affect the overall phosphorous removal efficiency.

The batch tests showed that the rate of anaerobic phosphorous release at different concentration of pCOD was similar. It was consistent with the results of SBRs performance, i.e. that the rate of anaerobic hydrolysis limited the availability of pCOD for PAO's activity in extended anaerobic conditions.

The results of SBRs simulation with low influent FCOD/TP showed that the calibrated model with default kinetic parameters of anaerobic hydrolysis in BioWin 4.1 could predict phosphorous

removal similar to that in experimental SBRs. With the default values of anaerobic hydrolysis in ASM2d, the model predicted significant hydrolysis of pCOD and overestimated phosphorous removal in the reactor with longer anaerobic HRT.

5 Chapter 5: Potential of hydrolysis/fermentation of activated sludge in sludge holding tank to improve nutrient removal efficiency of wastewater treatment plant [4]

Abstract

The objective of this study was to assess the potential of hydrolysis/fermentation of activated sludge (AS) in sludge holding tank (SHT) to increase the biological nutrient removal (BNR) efficiency. The study was conducted in anaerobic batch tests using the BNR sludge (from a full-scale Westside process) and the mixture of BNR sludge with conventional non-BNR activated sludge (to have higher biodegradable particulate COD (bpCOD) in sludge). During the batch tests at different times the nutrient release, filtered COD (FCOD) production and polyphosphate (poly-P) and aerobic phosphorous uptake of sludge were measured. Using the BioWin 4.1 simulator, the anaerobic batch test of the BNR was simulated; also the efficiency of FCOD utilization for nutrient removal in a Westside process was predicted (to assess the effect of FCOD production and nutrient release in SHT on main stream BNR). In all batch tests, the aerobic phosphorous uptake of sludge increased during anaerobic condition up to the point when poly-P was completely utilized; afterwards, it decreased significantly. The BioWin simulation predicted comparable results during first 12 h of anaerobic condition, but failed to predict the significant loss of aerobic phosphorous at longer time when poly-P was depleted. The COD production during hydrolysis/fermentation in the test with only BNR sludge was not enough for complete removal of nutrients released. In the test using mixed sludge, FCOD production was relatively higher. In case of activated sludge with relatively higher bpCOD (originating from a

[4] In process to be sibmitted for publication

plant with short SRT or without primary sedimentation), beneficial effect of SHT on BNR performance was found feasible. Results showed that a relatively low retention time and high sludge load in SHT could increase its potential to enhance BNR efficiency.

5.1 Introduction

The treatment of sludge produced in wastewater treatment process could contribute up to 60% of total operating cost of wastewater treatment plants (WWTP)(Yang et al., 2011). It has been shown that long-term starvation condition (no external electron acceptor or donor) in activated sludge process could reduce the sludge production in the system (Chon et al., 2011; Rodriguez-Perez and Fermoso, 2016). This method compared with other alternatives does not require extra chemical or physical steps; thus, could provide a cost effective and sustainable solution for sludge reduction. To use this in WWTP, a portion or entire of activated sludge (depending on available tankage) is sent to a basin with anaerobic/anoxic conditions (oxidation reduction potential (ORP) usually ranged from 100 to -250 mV) and returned back to bioreactor (Semblante et al., 2014). The unit in which sludge is kept under starvation condition is referred as sludge holding tank abbreviated SHT (Chen et al., 2003; Zhou et al., 2015).

The use of SHT for sludge reduction should not compromise the treatment efficiency of WWTP. Studies have reported 15-25% of sludge reduction using SHT, while the sludge settling property and chemical oxygen demand (COD) and nitrogen removal performance of treatment process were not negatively affected (in some cases improved) (Coma et al., 2013; Datta et al., 2009; Jönsson and Jansen, 2006; Rodriguez-Perez and Fermoso, 2016; Saby et al., 2003). The findings of studies on the effect of SHT on phosphorous removal are not conclusive (Semblante et al., 2014). Some studies reported that application of SHT could enhanced phosphorus removal efficiency (Chudoba et al., 1992; Gao et al., 2011; Goel and Noguera, 2006; Ye et al., 2007).

Huang & Goel (2015) observed similar phosphorous removal in sequencing batch reactors (SBR) with and without SHT. In some studies when the ORP in SHT was reduced (Saby et al., 2003) or aeration time and ratio of carbon to phosphorous in influent changed (to enhance phosphorous removal) (Datta et al., 2009), the SBR with SHT had lower phosphorous removal compared with the control.

Despite uncertainty about the effect of SHT on phosphorous removal, hydrolysis/fermentation of activated sludge (similar to SHT's condition) has been used in full-scale practices. Barnard et al. (2012) reported that fermentation of mixed liquor in a full-scale plant (Johannesburg type-BNR process) with relatively low SRT and no primary clarifier improved phosphorous removal. In about 30 Danish WWTP with relatively low VFA in the influent, the anaerobic basin was replaced with SHT to perform hydrolysis/fermentation of return activated sludge (RAS) (Jönsson and Jansen, 2006; Vollertsen et al., 2006).This enabled the plants to gain stable biological phosphorous removal.

The main mechanism through which SHT could enhance phosphorous removal is that the product of hydrolysis/fermentation of activated sludge could provide additional carbon for main stream BNR process (Kobylinski et al., 2013; Vollertsen et al., 2006). Different studies observed the VFA production in fermentation of activated sludge (Barnard et al., 2012; Jönsson and Jansen, 2006; Liu et al., 2009; Ucisik and Henze, 2008; Yuan et al., 2006) and some reported the nutrient release during the process (Chen et al., 2007; Ucisik and Henze, 2008; Yuan et al., 2010; Yuan et al., 2011), but the effect of nutrient release on mainstream BNR performance was not investigated. In the studies that the SHT was used with bioreactor, the nutrients were recovered from SHT's effluent (Li et al., 2011; Yan et al., 2015; Yuan and Oleszkiewicz, 2010). Houweling et al. (2010) estimated in theory the effect of nutrient release on overall carbon

production of activated sludge fermentation. Their results showed positive carbon production; however, as noted by authors, the calculations were based on the biomass composition only consisted of ordinary heterotrophs organisms (OHO) (conventional activated sludge); and phosphorus release by phosphorous accumulating organisms (PAO) was not accounted for.

The objective of this study was to assess the potential of hydrolysis/fermentation of activated sludge (application of SHT) to produce additional carbon for BNR processes. The study was conducted in batch tests of the hydrolysis/fermentation of activated sludge. Since the biodegradable particulate COD (bpCOD) in the sludge play an important role in hydrolysis/fermentation process, the investigation was performed on the sludge with different bpCOD concentration. To assess the effect of SHT on BNR performance, the effect of released nutrient during hydrolysis/fermentation on the BNR was estimated. The anaerobic batch tests with BNR sludge were simulated (dynamic simulation) in BioWin 4.1 and results were compared with the results of the experiment. Through this, the reliability of activated sludge modelling to predict the effect of SHT on BNR process was evaluated.

5.2 Material and Methods

5.2.1 **Batch tests**

The BNR sludge and CAS used in the batch tests were taken from West End Water Pollution Control center (WEWPCC) and South End Water Pollution Control Center (SEWPCC) which are located in Winnipeg, Canada, respectively. The WEWPCC is a BNR facility with 5-stage Westside process working at SRT of 10 days. The plant has primary sludge thickener-fermenter to provide additional carbon for BNR process. The SEWPCC is a carbon removal plant (high purity oxygen process) with SRT of 2.5 days.

The activated sludge was kept under anaerobic conditions in a batch reactor to stimulate the hydrolysis/fermentation process and sludge was collected at different times for analysis (Figure 5-1). The anaerobic batch reactor had working volume of 12 L and was completely mixed during the tests. Tests were conducted with BNR sludge alone or a mixture of BNR sludge and CAS (mixed sludge) to have higher bpCOD content in the sludge. The concentration of volatile suspended solids (VSS) in batch tests was about 6 g L^{-1}. The test with BNR sludge was conducted in triplicate and the reactor was sampled at times 0, 12, 24, and 48 h. For the batch test of mixed sludge, BNR sludge (42%) was mixed with CAS (58%) (mass of VSS). The batch reactor with mixed sludge was sampled at times 0, 12 and 24 h. At each sampling time, 1.5 L of sludge was collected and used for following analysis: poly-P concentration and aerobic phosphorous uptake of sludge, total suspended solids (TSS), VSS, filtered COD (FCOD), volatile fatty acids (VFA), NH_4^+-N, and PO_4^{-3}-P. The anaerobic batch reactors (for hydrolysis/fermentation of sludge) and the tests of measuring the poly-P and aerobic phosphorous uptake of sludge were conducted at 22±1 ℃.

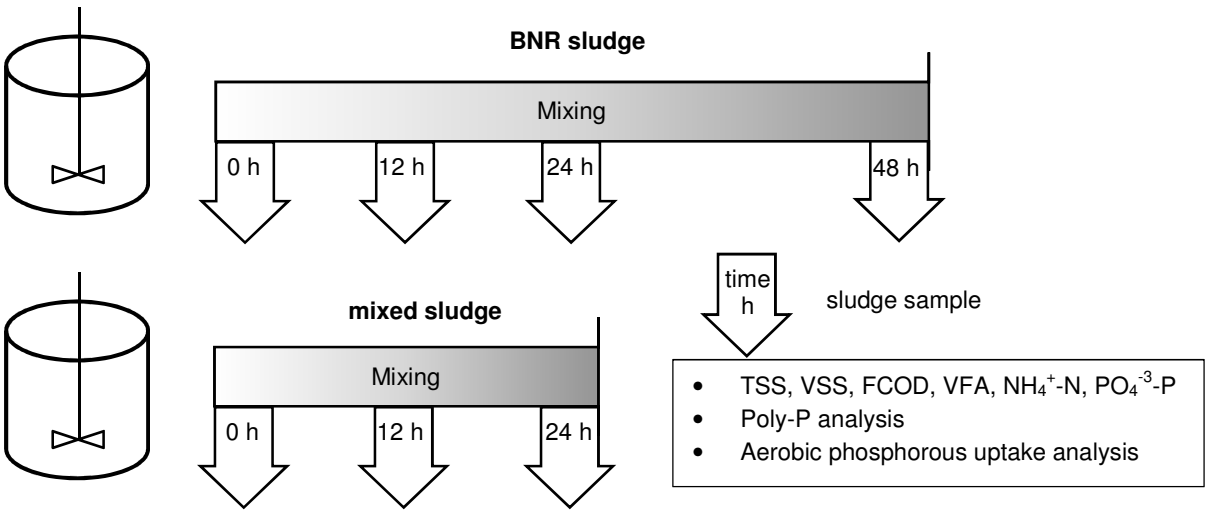


Figure 5-1 Schematic diagram of experimental design for hydrolysis/fermentation batch tests

5.2.2 **Poly-P storage analysis**

The 0.25 L of sludge was mixed with 0.25 L of mineral solution (described in Lopez et al. 2006) and was transferred to a batch reactor. The anaerobic condition was maintained in the batch reactor by purging N_2. At the beginning of test (time 0), 160 mg COD L^{-1} of acetate was added to the reactor and concentrations of PO_4^{-3}-P and FCOD during 2 h anaerobic condition (every 15 min) was measured. The pH was controlled at 7±0.1 by 0.5 M HCl or 0.5 M NaOH addition. This test was conducted in duplicate, in parallel with a control reactor (no acetate addition). The average of phosphorous release in the tests with acetate addition minus that in control accounted for the releasable poly-P storage of the sludge sample (Mino et al., 1985). The poly-P storage of BNR sludge (at time 0) was 29 mg P $gVSS^{-1}$; the CAS did not have excessive anaerobic phosphorous release with acetate addition compared with control (poly-P=0).

5.2.3 **Aerobic phosphorous uptake analysis**

The 0.25 L of sludge was mixed with 0.25 L of mineral solution and was transferred to a batch reactor. The aeration in the reactor maintained the concentration of dissolved oxygen (DO) above 4 mg L^{-1}. At the beginning of test (time 0), 50 mg PO_4^{-3}-P L^{-1} (in form of K_2HPO_4) was added to the reactor and concentration of PO_4^{-3}-P during 6 h aerobic conditions (every 30 min) was measured. The pH was controlled at 7±0.1 by 0.5 M HCl or 0.5 M NaOH addition. This test was conducted in duplicate. The aerobic phosphorous uptake of BNR sludge (at time zero) was 3.5±2.1 mg PO_4^{-3}-P $gVSS^{-1}$; the CAS did not exhibit aerobic phosphorous uptake.

The effect of hydrolysis/fermentation in the batch reactor on phosphorous removal of activated sludge at each sampling time was determined by calculating $net\ P\ removal_{t=HRT} = aerobic\ P\ uptake_{t=HRT} - (anaerobic\ P\ release_{t=HRT} + aerobic\ P\ uptake_{t=0})$.

5.2.4 **Analysis**

The samples for analysis of FCOD, NH_4^+-N, NO_3^--N, NO_2^--N and PO_4^{-3}-P were filtered through 0.45 µm filter. The FCOD was measured using Hach test kits. The PO_4^{-3}-P and NH_4^+-N were measured by Lachat Instruments (Ontario, Canada) Quik Chem 8500, according to orthophosphate method 10-115-01-1-9 O and ammonia method, respectively. For VFA analysis, samples were filtered through 0.2 µm filter and measured with Waters Breeze 2 HPLC system (Waters, Milford, MA, USA) with Aminex HPX-87H column with and 5 mM $H_2 SO_4$ as mobile phase. The concentration of MLSS and MLVSS were measured according to Standard Methods (APHA, 2005).

5.2.5 **BioWin Simulation**

5.2.5.1 Steady state simulation of BNR process

BioWin 4.1 Simulator (Envirosim Inc., Ontario, Canada) was used to predict the effect of hydrolysis/fermentation conditions on phosphorous removal of BNR sludge. A BNR process was simulated to produce activated sludge with VSS, poly-P and aerobic phosphorous uptake similar to the BNR sludge used in the experiment. The simulated BNR process was 5-stage Westside similar to the process at the WEWPCC – the source of the biomass (Figure 5-2). The modeled process included a thickener-fermenter for primary sludge (similar to WEWPCC) to provide additional VFA for BNR and increase the phosphorous removal efficiency (high PAO population /poly-P content in sludge). The batch reactor in return activated sludge (RAS) stream was by-passed in this simulation. The Wastewater characteristics (except for total phosphorous (TP)) and operational parameters were selected similar to those in WEWPCC. The TP in the influent, recycle ratio of activated sludge and SRT of the process (12 d compared with 10 d in WEWPCC) were adjusted to produce return activated sludge (RAS) with similar VSS (6 g L^{-1}), releasable

poly-P (29 mg PO_4^{-3}-P $gVSS^{-1}$) and aerobic phosphorous uptake (3 mg PO_4^{-3}-P $gVSS^{-1}$) of the BNR sludge used in experiment. The details of wastewater characteristics and kinetic and stoichiometric parameters used in the model are presented in Appendix-1.

The efficiency of FCOD utilization for nitrogen and phosphorous removal in the Westside process was determined in a steady state simulation. The batch reactor was by-passed in the simulation and the stream phosphorous addition was changed to COD addition. The composition of COD was readily biodegradable COD (rbCOD). The reduction in mg NO_3^--N and PO_4^{-3}-P in effluent compared control simulation (load of COD addition= 0) was used to determine the mg of PO_4^{-3}-P and NO_3^--N removed per mg of FCOD added to the process.

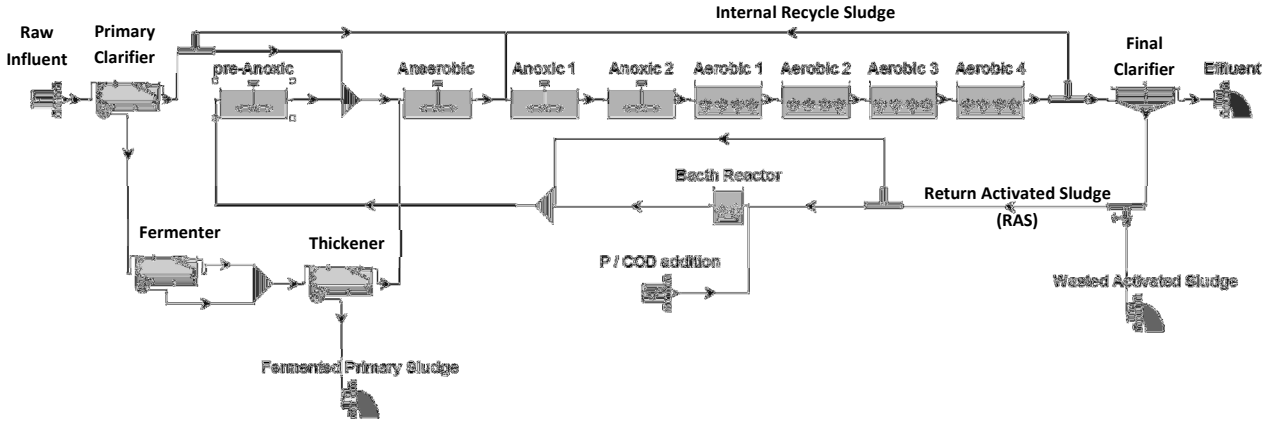


Figure 5-2 Schematic flow diagram of Westside process and the anaerobic batch reactor in BioWin simulation

5.2.5.2 Dynamic simulation of anaerobic batch tests

The anaerobic batch test of BNR sludge was simulated using the unit "variable volume reactor" of the simulator (named batch reactor in Figure 5-2). The BioWin's model was calibrated using the results of poly-P utilization and FCOD production in the batch test with BNR sludge. The

operation of the batch reactor, calibration method and details of kinetic and stoichiometric parameters used in the model are presented in Appendix-2.

5.3 Results and Discussions

5.3.1 **Results of anaerobic batch tests**

The results of anaerobic batch test with BNR sludge and profile of poly-P, aerobic phosphorous uptake and net phosphorous removal (defined in Methods) during anaerobic conditions are presented in Figure 5-3. The FCOD production and nutrient release in this manuscript are calculated based on the concentration of the parameter at each time minus its concentration at time zero. During the period of h 0 - 24, phosphorous release was 31.2 ± 3.1 mg PO_4^{-3}-P $gVSS^{-1}$; simultaneously, 26.4 ± 1.4 mg P $gVSS^{-1}$ of poly-P was utilized. During the period of h 24 - 48, phosphorous release decreased to 7.2 ± 2.1mg PO_4^{-3}-P $gVSS^{-1}$ and poly-P was completely consumed during this time. The phosphorous released in BNR sludge was because of poly-P utilization for carbon storage and activated sludge decay (Hao et al., 2010b). The relatively higher phosphorous release during h 0-24 was because of poly-P utilization to store VFA and low molecular soluble organic carbon became available through hydrolysis/fermentation of bpCOD (Kampas et al., 2009). When poly-P was completely consumed (at about h 24), phosphorous release was due to the low rate decay process in activated sludge.

The ammonium release was 2.4 ± 0.2 mg NH_4^+-N $gVSS^{-1}$ during h 0-12; afterwards (h 12-48), it increased to 6.5 ± 0.7 mg NH_4^+-N $gVSS^{-1}$. The ammonium release under anaerobic condition was because of ammonification of soluble organic nitrogen that is produced through activated sludge decay. The increase in ammonium release could be because the rates of hydrolysis/ biomass decay increased with the decrease of ORP during anaerobic condition(Chen et al., 2003; Saby et al., 2003). The profile of FCOD production shows that its rate steadily increased during

anaerobic condition. The FCOD was produced through hydrolysis of pCOD (exists initially in sludge or produced because of sludge decay under anaerobic condition). The increase in the rate of FCOD production could be because the rate of hydrolysis/decay process increased during anaerobic condition and the fact that after depletion of poly-P (about h 24), carbon was no longer stored and accumulates in the system.

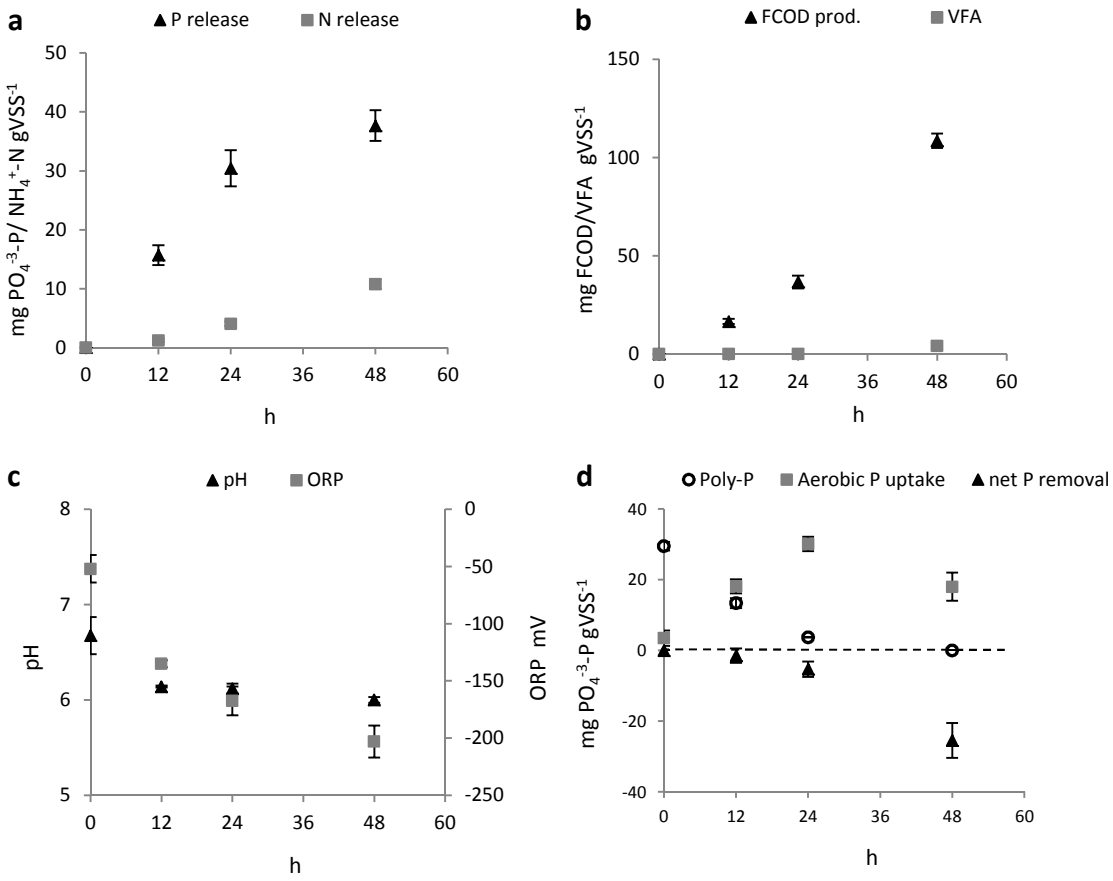


Figure 5-3 Results of a) phosphate and ammonium release; b) FCOD and VFA production, c) pH and ORP, d) poly-P, aerobic phosphorous uptake and net phosphorous removal in batch test with BNR sludge.

The aerobic phosphorous uptake of activated sludge increased during h 0-24 from 3 to 30 mg PO_4^{-3}-P $gVSS^{-1}$; but during h 24-48, it decreased by 40%. During anaerobic condition, bpCOD

was continuously converted to VFA (and other forms of FCOD that could be used by PAO) through hydrolysis/fermentation and stored by PAO; this increased the capacity of biomass for phosphorous uptake under aerobic conditions. The reason for reduction of aerobic phosphorus uptake will be discussed later. The net phosphorous removal was negative at all tested anaerobic time. This showed that the aerobic phosphorous uptake capacity attained during hydrolysis/fermentation was not sufficient for removing the sum of phosphorous released because of poly-P usage and biomass decay during the same period. The net phosphorous removal decreased at higher rate when the poly-P storage was completely depleted (after h 24). It was because the phosphorous was continuously released due to sludge decay, while aerobic phosphorous uptake decreased during this time.

The type of activated sludge could affect the FCOD production and the ratio of VFA/FCOD produced through hydrolysis/fermentation processes (Ucisik and Henze, 2008). The content of bpCOD in activated sludge is the main source for hydrolysis/fermentation process (Foladori et al., 2015). The bpCOD in activated sludge decreases with SRT increase in the treatment process (Eliosov and Argaman, 1995). As result, less FCOD (and VFA) would be produced during hydrolysis/fermentation condition. It could be reasoned that a different BNR activated sludge with relatively higher bpCOD (no primary sedimentation and relatively lower SRT of process) could provide higher FCOD concentration during anaerobic condition. Therefore, a positive effect of hydrolysis/fermentation of sludge (SHT application) on BNR performance might be achieved. The results of batch test with mixed sludge that had higher bpCOD than BNR sludge are presented in Figure 5-4.

During the period of h 0 to 12, phosphorous release was 40.8 mg PO_4^{-3}-P $gVSS^{-1}$; simultaneously 33.6 mg P $gVSS^{-1}$ of poly-P was utilized (Figure 5-4d). In the period of h 12-24, phosphorous

release decreased to 4.1 mg PO_4^{-3}-P $gVSS^{-1}$ and poly-P was completely depleted. In this test, and similar to the test with BNR sludge, the anaerobic phosphorous release significantly dropped after poly-P depletion. The FCOD production at h 12 and 24 were 48 and 83 mg COD $gVSS^{-1}$, respectively; these are significantly higher than 17 ± 1 and 37± 3 mg $gVSS^{-1}$ in the test with BNR sludge at similar times. The VFA accumulated at low concentration (6 to 8 mg L^{-1}) in the test with mixed sludge; while, no VFA accumulated in the test using BNR sludge. This confirmed the hypothesis that the sludge with relatively higher bpCOD could produce more carbon during hydrolysis/fermentation process.

The aerobic phosphorous uptake increased until h 12; afterwards, it decreased by 13%. The poly-P was almost depleted (2 mg PO_4^{-3}-P $gVSS^{-1}$) at h 12. These results are consistent with results of the test with BNR sludge (Figure 5-3d) that the aerobic phosphorous uptake decreased after poly-P was completely utilized. The net phosphorous removal in the test with mixed sludge at h 12 and 24 were 2 and -3 mg PO_4^{-3}-P $gVSS^{-1}$, respectively. It is consistent with the results of the test with BNR sludge that the net phosphorus removal decreased with time under anaerobic conditions. Since the net phosphorous removal at h 24 became negative test was terminated.

In the hydrolysis/fermentation batch tests of both BNR and mixed sludge, the aerobic phosphorous uptake decreased significantly after poly-P was depleted. In these batch tests, the carbon for PAO was produced through hydrolysis of bpCOD. The rate of hydrolysis process ,in particular under anaerobic condition, is relatively low (Eastman and Ferguson, 1981; Morgenroth et al., 2002); thus, carbon became available at relatively low rate to PAO compared with that in mainstream anaerobic basin. This causes that the time required in activated sludge hydrolysis/fermentation process to provide carbon for PAO (0.5-2 d, (Vollertsen et al. 2006)) is significantly longer than that in main stream anaerobic basin (0.5- 1.5 h, (Grady et al., 2011)).

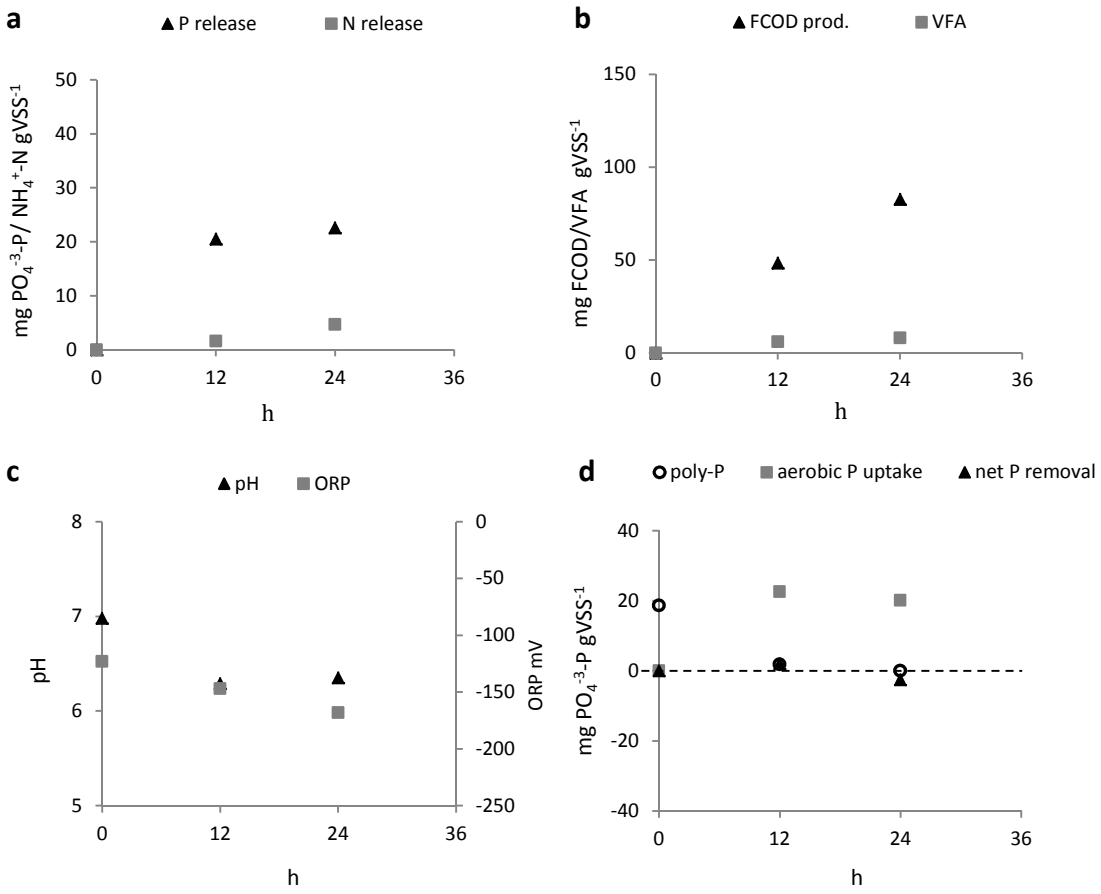


Figure 5-4 The results of a) phosphate and ammonium release; b) FCOD and VFA production, c) pH and ORP, d) poly-P, aerobic phosphorous uptake and net phosphorous removal in batch test with mixed sludge.

The longer retention time under anaerobic condition results that biomass is maintained under starvation condition. It has been reported that under anaerobic starvation condition, PAO use their internal storage of glycogen and poly-P simultaneously, to produce maintenance energy; also the composition of PHA could change from poly-b-hydroxybutyrate (PHB) to poly-b-hydroxyvalerate (PHV) (Lu et al., 2007; Vargas et al., 2013; Wang et al., 2012). Vargas et al (2013) reported that under starvation conditions (cycles with 5.75 h of anaerobic and 0.25 h of aerobic condition), the poly-P utilization efficiency for carbon storage (phosphorous released per acetate up-taken) and VFA sequestration efficiency (PHA stored per acetate up-taken) decreased

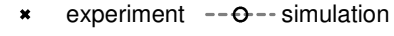
with starvation time, but PAO were able to recover after several cycles when carbon was provided. They concluded that the temporary loss of metabolic activity rather than cell death occurs under anaerobic starvation. Lu et al. (2007) proposed that under anaerobic starvation, when glycogen is reduced to certain level, PAO activate another metabolic pathway through which PHB is converted to glycogen and subsequently converted to adenosine-triphosphate (ATP) for producing energy and PHV as by-product. In their study, PAO's decay under anaerobic conditions was negligible. The findings of these studies suggest that under anaerobic starvation the change in metabolic pathways (adaptation to starvation condition) rather than cell death is responsible for reduction in PAO's activity. The change in metabolic pathway of PAO would cause PHB conversion to PHV. It has been shown that the efficiency of phosphorous uptake (per PHA utilized) for PHV is lower than that for PHB (Chen et al., 2004; Pijuan et al., 2004; Randall and Liu, 2002). Therefore, the starvation condition could reduce the aerobic phosphorous uptake ability of activated sludge. This could explain the loss of aerobic phosphorous uptake in this study when poly-P was depleted in the sludge (h 24-48 in the test with BNR sludge (Figure 5-3d) and h 12-24 in the test with mixed sludge (Figure 5-4d)). Lopez et al. (2006) also observed that under starvation condition, the rate of aerobic phosphorous uptake of PAO decreased after poly-P depletion.

5.3.2 **Results of BioWin simulation**

The calibrated model was used in dynamic simulation to assess the effect of hydrolysis/fermentation of BNR sludge on its phosphorous removal performance. The results of the simulation and experiment are compared in Figure 5-5. The profile of poly-P in simulation at all anaerobic times was similar to the experiment because the model was calibrated accordingly. During the period of h 0 – 12, the model predicted similar anaerobic phosphorous release and

aerobic phosphorous uptake to those in the experiment; as a result the net phosphorous removal in simulation and experiment were comparable. During this period, the anaerobic phosphorous release was due to poly-P utilization and sludge decay. Since the model was calibrated based on poly-P utilization; it was concluded that the model could predict the phosphorous release due to sludge decay accurately. This conclusion could also be drawn for the period of h 12-24 (similar anaerobic phosphorous release and poly-P utilization). However for this period, the aerobic phosphorous uptake in simulation increased 60% more than that in the experiment. The higher aerobic phosphorous uptake predicted in simulation led to the net phosphorous removal being overestimated for this period.

During the period of h 24-48, poly-P was completely depleted in both simulation and experiment. During this time, the anaerobic phosphorous release (due to sludge decay) in experiment was slightly higher than that in the simulation. It could be because the rate of sludge decay in the experiment increased with time and reduction of ORP (consistent with the increase of ammonium release in the experiment, Figure 5-3a); while, the model considers constant rate of sludge decay regardless of ORP value and starvation time. The aerobic phosphorous uptake for the period of h 24-48 in simulation was almost constant, but in the experiment it decreased 40%. The model considers the PHA lysis/PAO decay at relatively low rate under starvation condition (slight reduction in aerobic phosphorous uptake during h 36- 48 in simulation, Figure 5-5c); this clearly could not make up for significant reduction of aerobic phosphorous uptake in the experiment. During the period of h 24-48, the net phosphorous removal significantly dropped in the experiment; while in the simulation, it slightly decreased (aerobic phosphorous uptake rate was constant, while phosphorous was continuously released).



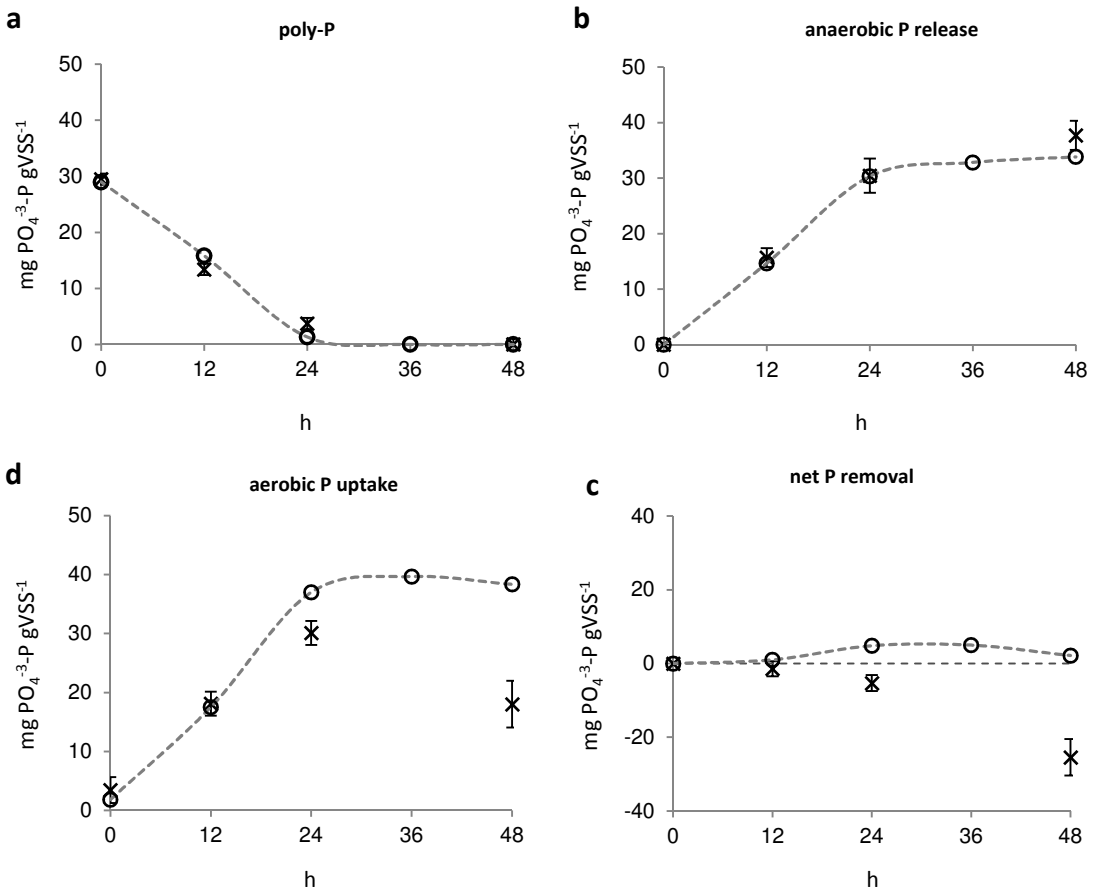


Figure 5-5 Comparison of results of a) anaerobic phosphorous release, b) poly-P, c) aerobic phosphorous uptake and d) net phosphorous removal in the batch tests with BNR sludge and simulation

The rate of PAO decay (and associated PHA lysis) under anaerobic condition in the model could not account for the loss of aerobic phosphorous uptake measured in the experiment. Increasing the rate of PAO decay (and PHA lysis) to calibrate the model might not be the proper method. It is because in the BioWin's model (similar to ASM2d based models) the PHA lysis due to the decay of PAO produces VFA as by-product; while, the possible cause for the loss of aerobic phosphorous uptake (change in PAO's metabolic pathway and conversion of PHB to PHV) does not result in carbon production and reduces the net phosphorous removal of SHT. Besides, several studies have shown the PAO decay under starvation condition is negligible (Foladori et

al., 2015; Lu et al., 2007; Vargas et al., 2013). For future studies on modelling of activated sludge under starvation condition (SHT modelling), the maintenance approach rather than PAO decay under anaerobic conditions should be used (Houweling et al., 2010; Lopez et al., 2006). The current equation for maintenance approach only considers use of poly-P for energy production, but PHA storage is not affected (Smolders et al., 1995). This could not predict the loss of aerobic phosphorous uptake due to anaerobic starvation. Therefore, the model should be further modified to consider the negative effect of starvation condition on aerobic phosphorous uptake of PAOs e.g. two type PHA compositions with different aerobic phosphorous uptake efficiency.

5.3.3 **Effect of COD production and nutrient release on BNR**

The experimental results showed that hydrolysis/fermentation of the BNR sludge could not produce positive net phosphorous removal. In order to determine the overall effect hydrolysis/fermentation of activated sludge on BNR performance, the effect of FCOD production and nitrogen release should also be accounted for. In the batch test, the concentration of FCOD and ammonium continuously increased during anaerobic condition. The answer to whether the produced FCOD is sufficient (or exceeds the need) for removal of nitrogen and phosphorous (in case of negative net phosphorous removal) released depends on efficiency of FCOD utilization for nutrient removal in main stream BNR process. For example, the process with internal sludge recycle from aerobic to anoxic tank performs simultaneous phosphorous and nitrogen removal (denitrifying PAO activity) that lowers the carbon required for denitrification and phosphorous removal. For the BNR process simulated in this study (5-stage Westside process) the reduction in effluent's phosphorous and nitrogen concentration due to FCOD produced were estimated 0.08 mg PO_4^{-3}-P $mgCOD^{-1}$ and 0.03 mg NO_3^{-}-N $mgCOD^{-1}$,

respectively (explained in Methods). Using these ratios, the effect of FCOD production and nutrient release in the tests with BNR on main stream BNR performance was estimated. It was assumed that all nitrogen release is oxidized to nitrate and all FCOD are readily biodegradable; the results are presented in Table 5-1 Effect of FCOD production and nutrient release in test with BNR sludge on BNR performance.

Table 5-1 Effect of FCOD production and nutrient release in test with BNR sludge on BNR performance

anaerobic time	FCOD prod.	Net P removal	N release	P removal due to FCOD prod.[a]	N removal due to FCOD prod.[b]	Total P removal[c]	Total N removal[d]
h	mg COD $gVSS^{-1}$	mg PO_4^{-3}-P $gVSS^{-1}$	mg NO_3^--N $gVSS^{-1}$	mg PO_4^{-3}-P $gVSS^{-1}$	mg NO_3^--N $gVSS^{-1}$	mg PO_4^{-3}-P $gVSS^{-1}$	mg NO_3^--N $gVSS^{-1}$
12	16	-1.4	1.1	1.0	0.5	-0.4	-0.5
24	37	-5.3	4.3	2.6	1.4	-2.7	-2.9
48	110	-25.4	11.6	7.2	3.9	-18.3	-7.7

[a] The P removal due to FCOD produced is calculated as FCOD production timed by 0.08 gPO_4^{-3}-P $gCOD^{-1}$.

[b] The N removal due to FCOD produced is calculated as FCOD production timed by 0.03 gNO_3^--N $gCOD^{-1}$.

[c] Total P removal is calculated as net P removal plus P removal due to FCOD prod.

[d] Total N removal is calculated as N removal due to FCOD prod. minus N released.

For the BNR sludge, the FCOD produced at all sampling times could not provide required carbon for complete removal of nutrient released during the same period (negative total nitrogen and phosphorous removal). At h 12, the total nitrogen and phosphorous removal were close to zero; but at a longer anaerobic time, the carbon deficiency increased despite higher FCOD production. The test with mixed sludge had higher FCOD production, while the net phosphorous removal and nitrogen release were similar during similar period. Thus, for sludge that has relatively high bpCOD (e.g. low operational SRT and no primary sedimentation in treatment

plant), the positive effect of activated sludge hydrolysis/fermentation in SHT on total BNR performance of WWTP is feasible.

The use of SHT in BNR process could influence the microbial community of activated sludge (Chudoba et al., 1992; Ning et al., 2014; Ye et al., 2007). Stokholm-Bjerregaard et al. (2015) reported that treatment plants with SHT had relatively lower population of GAO. The batch tests of their study also showed that PAO compared with GAO could sustain their acetate uptake activity for longer time under starvation conditions. Based on these findings they suggested that the starvation conditions in SHT could give competitive advantages to PAO over GAO. The study by Tu et al. (2013) showed that adding acetate at low concentration under anaerobic conditions (similar to SHT' condition) promoted PAO's growth over GAO. If SHT could results in higher ratio of PAO to GAO in activated sludge system, it would increase the efficiency of carbon utilization for nutrient removal and consequently improve the BNR performance. However, Vargas et al.(2013) found that during first few days of starvation (alternating anaerobic/aerobic conditions), the cell death and loss of carbon storage activity of PAO were higher than those of GAO. The effect of SHT on PAO-GAO competition needs to be further investigated.

The results of this study suggest that the high VFA concentration in SHT may not necessarily increase its potential to improve BNR performance. The high VFA concentration indicates that the poly-P storage in sludge has been completely depleted (no further carbon storage) and the biomass stays under starvation condition. This could significantly reduce the aerobic phosphorous uptake capacity of sludge; while, the FCOD production at such long starvation condition may not compensate for the nutrient release and loss of aerobic phosphorous uptake

(Table 1). Therefore to increase the potential of SHT to improve the BNR efficiency of WWTP, a relatively low retention time with higher sludge load is recommended.

5.4 Conclusion

The batch tests with BNR sludge showed that the aerobic phosphorous uptake capacity obtained by biomass during hydrolysis/fermentation was lower than the mass of phosphorous released (negative net phosphorous removal). This was mainly because the aerobic phosphorous uptake deteriorated after poly-P depletion in the sludge (negative effect of starvation conditions). In the batch test using mixed sludge (relatively higher bpCOD content than BNR sludge), the FCOD production increased, but net phosphorus removal became negative at h 24 because of reduction in aerobic phosphorous uptake (coincident with poly-P depletion).

The calibrated model predicted similar anaerobic phosphorous release and aerobic phosphorous uptake to those in the experiment during the period of h 0-12; afterwards, the model could not account for significant loss of aerobic phosphorous uptake caused by starvation conditions on PAO's metabolism in the experiment. For modelling of activated sludge under hydrolysis/fermentation conditions (modelling of SHT in WWTP), the model should consider maintenance approach rather than decay for PAO under anaerobic conditions. In addition, the maintenance approach has to be modified to account for the negative effect of starvation conditions on aerobic phosphorous uptake of sludge.

In the batch test with BNR sludge, the FCOD produced during hydrolysis/fermentation could not provide required carbon for complete removal of nutrients released during that period (negative total nitrogen and phosphorous removal). For the sludge with relatively high bpCOD (e.g. low

operational SRT and no primary sedimentation in WWTP), hydrolysis/fermentation of activated sludge (SHT application) could potentially produce additional carbon for BNR process.

The results of this study concluded that in design and operation of SHT in WWTP, a relatively low retention time with higher sludge load could increase the potential of SHT to improve the BNR efficiency of WWTP.

6 Chapter 6: Main findings, contribution to wastewater treatment engineering and recommendations for future studies

The removal of phosphorous and nitrogen from wastewater is critical to protect the ecosystem of water bodies that receive the WWTP's effluent. The most sustainable treatment methods are biological nutrient removal (BNR) processes. These bioprocesses are conducted by certain type of microbial groups that require carbon source to fulfil the treatment processes. The stringent effluent standards imposed by regulatory authorities require that these processes have high treatment efficiency; as a result a significant amount of carbon is required. The carbon matter that is readily available in wastewater is usually not enough for BNR processes to meet the effluent standards. One common practice is to add external carbon source such as methanol or acetate. But, this is not desired because it increases the carbon footprint of WWTPs and negatively affects the sustainability of the treatment facility.

The objective of this thesis was to examine the feasibility of following alternatives to reduce the carbon demand of BNR process:

1. Simultaneous denitritation and phosphorous removal in IFAS system
2. Hydrolysis of particulate COD under extended anaerobic condition to enhance EBPR
3. Hydrolysis/fermentation of activated sludge in SHT to enhance BNR efficiency.

In the following sections for each investigated alternative, the main findings and contribution to wastewater treatment engineering and recommendation for future studies are presented.

6.1 Simultaneous denitritation and phosphorous removal in IFAS system

6.1.1 **Main findings and contribution**

In this research it was demonstrated that the long term simultaneous denitritation and phosphorous removal could be achieved by DPAO in both suspended and attached biomass. The free nitrous acid (FNA) at relatively high concentrations up to 1.6 μg HNO_2-N L^{-1} did not inhibit DPAO's activity. The attached biomass was found to be more resistant to FNA inhibition compared with suspended biomass in IFAS system.

Some values obtained in this experiment could be used for process modelling of BNR –IFAS systems. The contribution of suspended and attached biomass into phosphorous removal and nitritation in IFAS system was quantified for the first time. The FNA inhibition threshold of aerobic/anoxic phosphorous removal in IFAS system was quantified for the first time with known level of biomass acclimation to FNA. These values could be used in activated sludge modeling of BNR-IFAS systems that are prone to nitrite accumulation.

6.1.2 **Future studies**

1) The results from this research proved the feasibility of DPAO's activity with nitrite in IFAS system. In order to apply this enhanced BNR process in treatment system, the configuration of the treatment system needs to provide cyclic anaerobic/anoxic conditions. This could be achieved in SBR type IFAS systems (Garzón-Zúñiga and González-Martínez, 1996; Helness and Odegaard, 2001). For continuous flow treatment process, two SBR-IFAS bioreactors could be used in parallel, while their anoxic and anaerobic stages happen interchangeably. The nitritation process (to produce nitrite) could be conducted in separate bioreactor with relatively short aerobic retention time or

DO-controlled conditions (Liang et al., 2011). The development of such process configuration and its BNR performance could be a topic for future investigation.

2) In this study, the investigation was conducted in bioreactor using synthetic wastewater. It is recommended that the experiment is conducted with real wastewater in future studies, in this way the results could be used for pilot-scale design and modelling of the process.

3) In this study, AOB and DPAO that require respectively, high and low SRT were selected in attached biomass of IFAS system. In order to identify the operational strategy to select these organisms in biofilm, the effect of operational parameters on spatial distribution of AOB and DPAO within biofilm should be further investigated.

6.2 Hydrolysis of pCOD under extended anaerobic condition to enhance EBPR

6.2.1 **Main findings and contribution**

This research showed that the potential of extending anaerobic HRT to enhance EBPR efficiency is limited in reality, and the current kinetics of anaerobic hydrolysis used in activated sludge modeling practices could overestimate the phosphorous removal when anaerobic HRT is extended. In recent version of BioWin (4.0, 4.1 and 5.0) the kinetics of anaerobic hydrolysis is reduced significantly. However, many activated sludge modelling studies and commercial simulators (e.g. GPSX) use the default kinetics of ASM2d (Henze et al., 2007) for anaerobic hydrolysis. Thus, they are prone to overestimate the BNR efficiency in the case of long anaerobic HRT.

While the results of this study suggest that the rate of anaerobic hydrolysis is relatively lower than that estimated by ASM2d kinetics, it is recommend that in process modelling of full-scale

BNR-WWTPs, the kinetics of anaerobic hydrolysis for each specific wastewater is estimated experimentally. The long-term experimental methods used in Chapter 4 (SBR operation) could be used to estimate the kinetics of anaerobic hydrolysis.

In conclusion, the most significant findings of this investigation was that the kinetics of anaerobic hydrolysis used in activated sludge modelling needs to be re-evaluated; the current kinetics in commercial simulators may lead to underestimation of carbon demand of BNR process, especially in cases with relatively long anaerobic HRT.

6.2.2 **Future studies**

For future studies, the internal storages of poly-p phosphate, poly hydroxyalkanoates and glycogen in PAO in bioreactors with different anaerobic HRT could be a topic of investigation. These results could be used in calibration of activated sludge models, thus, the rate of anaerobic hydrolysis of pCOD could be determined more reliably.

Another topic for future study could be assessing the effect of protozoan and metazoan community in activated sludge on anaerobic hydrolysis processes. It is believed that these organisms play an important role in aerobic hydrolysis of pCOD but are less active during anaerobic conditions. This postulation needs further investigation, because for example, the study of Priya et al. (2007) has shown that ciliated protozoa play an important role in anaerobic digestion systems.

6.3 Hydrolysis/fermentation of activated sludge in SHT to enhance BNR efficiency

6.3.1 **Main findings and contribution**

The findings of the kinetic tests and BioWin simulation showed that the effect of SHT on BNR efficiency depends on the type of BNR sludge. This finding can be applied in WWTPs with low operational SRT and without primary sedimentation (so the biomass receives has high biodegradable pCOD) to apply the SHT to improve the BNR performance.

This research also demonstrated that the BioWin model (and all ASM2d-based models that are widely used for BNR process simulation in commercial wastewater treatment simulators) could not predict the effect of SHT on BNR performance. It is because the current activated sludge models fail to simulate the PAO's dynamic under starvation conditions; thus, they may overestimate the benefit of SHT on BNR efficiency.

This research also concluded that the retention of BNR sludge in SHT after poly-P depletion should be avoided or minimized. Therefore in design and operation of SHT, a relatively low retention time and higher sludge load could increase the potential of SHT to enhance BNR efficiency of WWTPs.

6.3.2 **Future studies**

The ASM2d-based activated sludge model (as BioWin model) needs to be modified in order to simulate the SHT in WWTPs. Based on the findings of this study, two modifications are recommended (maintenance approach instead of PAO's decay and two compositions for PHA with different efficiency for phosphorous uptake). In a perspective study, these changes could be implemented in the activated sludge model and the reliability of the modified model should be verified. The methodology presented in Chapter 5 can be used for model verification.

The effect of SHT on PAO-GAO competition in activated sludge community needs to be further investigated. This is critical to answer if SHT could lead to reliable phosphorous removal

performance in WWTPs. The current knowledge suggests that SHT favors the growth of PAO over GAO's growth, but to conclude, more investigation is required. One approach could be using the methodology proposed by López-Vázquez et al. (2007) to compare the PAO/GAO ratio in similar BNR processes with and without SHT.

Appendix A

Supporting data for Chapter 4

In this appendix the wastewater characteristics and kinetic and stoichiometric parameters used in for the model to simulate the SBRs are presented.

Wastewater characteristics used in simulation

parameters	unit	Abbreviation	raw wastewater (Default)	Input (calibrated)
rbCOD (including Acetate)	gCOD/g of total COD	Fbs	0.16	0.1[a]
Acetate	gCOD/g of readily biodegradable COD	Fac	0.15	0.15
Non-colloidal slowly biodegradable	gCOD/g of slowly degradable COD	Fxsp	0.75	0.75
Unbiodegradable soluble	gCOD/g of total COD)	Fus	0.05	0.07[a]
Unbiodegradable particulate	gCOD/g of total COD	Fup	0.26	0.13[a]
Ammonia	gNH3-N/gTKN	Fna	0.66	0.45[b]
Particulate organic nitrogen	gN/g Organic N	Fnox	0.5	0.5
Soluble unbiodegradable TKN	gN/gTKN	Fnus -	0.02	0.02
N:COD ratio for unbiodegradable part. COD	gN/gCOD	FupN	0.035	0.035
Phosphat	gPO4-P/gTP	Fpo4	0.5	0.56[b]
P:COD ratio for unbiodegradable part. COD	gP/gCOD	FupP	0.011	0.011
OHO COD fraction	gCOD/g of total COD	FZbh	0.02	0.02
AOB COD fraction	gCOD/g of total COD	FZaob	1.000E-4	1.000E-4
NOB COD fraction	gCOD/g of total COD	FZnob	1.000E-4	1.000E-4
PAO COD fraction	gCOD/g of total COD	FZbp	1.000E-4	1.000E-4
Endogenous products COD fraction	gCOD/g of total COD	FZe	0	0

[a] These values are calculated from average wastewater characteristics

[b] These values are estimated from the results of kinetic tests

BioWin simulation and model calibration

The SRT of SBRs in simulation was set at 8.3 d compared with 10 d in the experiment to account for the estimated biomass loss through regular sampling and solids in the effluent (TSS<40 mg L^{-1}). The DO profile during mixing (anaerobic/anoxic) and aeration stages were zero and 4.5 mg L^{-1} based on the DO profile measured in SBRs. The Yield of heterotrophs was adjusted to 0.5 for aerobic condition and 0.45 for anoxic condition to calibrate the MLVSS concentrations in the simulation to experimental values of 2020±170, 1820±150 and 1540±120 mg L^{-1} in SBR1, SBR2 and SBR3, respectively.

It should be noted that the simulations were conducted for 30 d which is longer than the simulated period (11 d). In simulations, the differences in characteristics of SBRs' effluent after 5 d were similar to those at the end of 30 d of simulation. Thus, the experimental and simulation results could be compared. The other kinetic and stoichiometric parameters were the default values in integrated model of BioWin 4.1 (Tables below).

Kinetic and stoichiometric parameters

Kinetic Parameters

Common

Name	Default	Value	Arrhenius
Hydrolysis rate [1/d]	2.1000	2.1000	1.0290
Hydrolysis half sat. [-]	0.0600	0.0600	1.0000
Anoxic hydrolysis factor [-]	0.2800	0.2800	1.0000
Anaerobic hydrolysis factor (AS) [-]	0.0400	0.0400	1.0000
Anaerobic hydrolysis factor (AD) [-]	0.5000	0.5000	1.0000
Adsorption rate of colloids [L/(mgCOD d)]	0.1500	0.1500	1.0290
Ammonification rate [L/(mgN d)]	0.0400	0.0400	1.0290
Assimilative nitrate/nitrite reduction rate [1/d]	0.5000	0.5000	1.0000
Endogenous products decay rate [1/d]	0	0	1.0000

AOB

Name	Default	Value	Arrhenius
Max. spec. growth rate [1/d]	0.9000	0.9000	1.0720
Substrate (NH4) half sat. [mgN/L]	0.7000	0.7000	1.0000
Byproduct NH4 logistic slope [-]	50.0000	50.0000	1.0000
Byproduct NH4 inflection point [mgN/L]	1.4000	1.4000	1.0000
AOB denite DO half sat. [mg/L]	0.1000	0.1000	1.0000
AOB denite HNO2 half sat. [mgN/L]	5.000E-6	5.000E-6	1.0000
Aerobic decay rate [1/d]	0.1700	0.1700	1.0290
Anoxic/anaerobic decay rate [1/d]	0.0800	0.0800	1.0290
KiHNO2 [mmol/L]	0.0050	0.0050	1.0000

NOB

Name	Default	Value	Arrhenius
Max. spec. growth rate [1/d]	0.7000	0.7000	1.0600
Substrate (NO2) half sat. [mgN/L]	0.1000	0.1000	1.0000
Aerobic decay rate [1/d]	0.1700	0.1700	1.0290
Anoxic/anaerobic decay rate [1/d]	0.0800	0.0800	1.0290
KiNH3 [mmol/L]	0.0750	0.0750	1.0000

OHO

Name	Default	Value	Arrhenius
Max. spec. growth rate [1/d]	3.2000	3.2000	1.0290
Substrate half sat. [mgCOD/L]	5.0000	5.0000	1.0000
Anoxic growth factor [-]	0.5000	0.5000	1.0000
Denite N2 producers (NO3 or NO2) [-]	0.5000	0.5000	1.0000
Aerobic decay rate [1/d]	0.6200	0.6200	1.0290
Anoxic decay rate [1/d]	0.2330	0.2330	1.0290
Anaerobic decay rate [1/d]	0.1310	0.1310	1.0290
Fermentation rate [1/d]	1.6000	1.6000	1.0290
Fermentation half sat. [mgCOD/L]	5.0000	5.0000	1.0000
Fermentation growth factor (AS) [-]	0.2500	0.2500	1.0000
Free nitrous acid inhibition [mmol/L]	1.000E-7	1.000E-7	1.0000

PAO

Name	Default	Value	Arrhenius
Max. spec. growth rate [1/d]	0.9500	0.9500	1.0000
Max. spec. growth rate, P-limited [1/d]	0.4200	0.4200	1.0000
Substrate half sat. [mgCOD(PHB)/mgCOD(Zbp)]	0.1000	0.1000	1.0000
Substrate half sat., P-limited [mgCOD(PHB)/mgCOD(Zbp)]	0.0500	0.0500	1.0000
Magnesium half sat. [mgMg/L]	0.1000	0.1000	1.0000
Cation half sat. [mmol/L]	0.1000	0.1000	1.0000
Calcium half sat. [mgCa/L]	0.1000	0.1000	1.0000
Aerobic/anoxic decay rate [1/d]	0.1000	0.1000	1.0000
Aerobic/anoxic maintenance rate [1/d]	0	0	1.0000
Anaerobic decay rate [1/d]	0.0400	0.0400	1.0000
Anaerobic maintenance rate [1/d]	0	0	1.0000
Sequestration rate [1/d]	4.5000	4.5000	1.0000
Anoxic growth factor [-]	0.3300	0.3300	1.0000

pH

Name	Default	Value
OHO low pH limit [-]	4.0000	4.0000
OHO high pH limit [-]	10.0000	10.0000
Methylotrophs low pH limit [-]	4.0000	4.0000
Methylotrophs high pH limit [-]	10.0000	10.0000
Autotrophs low pH limit [-]	5.5000	5.5000
Autotrophs high pH limit [-]	9.5000	9.5000
PAO low pH limit [-]	4.0000	4.0000
PAO high pH limit [-]	10.0000	10.0000
OHO low pH limit (anaerobic) [-]	5.5000	5.5000
OHO high pH limit (anaerobic) [-]	8.5000	8.5000
Propionic acetogens low pH limit [-]	4.0000	4.0000
Propionic acetogens high pH limit [-]	10.0000	10.0000
Acetoclastic methanogens low pH limit [-]	5.0000	5.0000
Acetoclastic methanogens high pH limit [-]	9.0000	9.0000
H2-utilizing methanogens low pH limit [-]	5.0000	5.0000
H2-utilizing methanogens high pH limit [-]	9.0000	9.0000

Switches

Name	Default	Value
Aerobic/anoxic DO half sat. [mgO2/L]	0.0500	0.0500
Anoxic/anaerobic NOx half sat. [mgN/L]	0.1500	0.1500
AOB DO half sat. [mgO2/L]	0.2500	0.2500
NOB DO half sat. [mgO2/L]	0.5000	0.5000
AAO DO half sat. [mgO2/L]	0.0100	0.0100
Anoxic NO3(->NO2) half sat. [mgN/L]	0.1000	0.1000
Anoxic NO3(->N2) half sat. [mgN/L]	0.0500	0.0500
Anoxic NO2(->N2) half sat. (mgN/L)	0.0100	0.0100
NH3 nutrient half sat. [mgN/L]	0.0050	0.0050
PolyP half sat. [mgP/mgCOD]	0.0100	0.0100
VFA sequestration half sat. [mgCOD/L]	5.0000	5.0000
P uptake half sat. [mgP/L]	0.1500	0.1500
P nutrient half sat. [mgP/L]	0.0010	0.0010
Autotroph CO2 half sat. [mmol/L]	0.1000	0.1000
H2 low/high half sat. [mgCOD/L]	1.0000	1.0000
Propionic acetogens H2 inhibition [mgCOD/L]	5.0000	5.0000
Synthesis anion/cation half sat. [meq/L]	0.0100	0.0100

Stoichiometric Parameters

Common

Name	Default	Value
Biomass volatile fraction (VSS/TSS)	0.9200	0.9200
Endogenous residue volatile fraction (VSS/TSS)	0.9200	0.9200
N in endogenous residue [mgN/mgCOD]	0.0700	0.0700
P in endogenous residue [mgP/mgCOD]	0.0220	0.0220
Endogenous residue COD:VSS ratio [mgCOD/mgVSS]	1.4200	1.4200
Particulate substrate COD:VSS ratio [mgCOD/mgVSS]	1.6000	1.6000
Particulate inert COD:VSS ratio [mgCOD/mgVSS]	1.6000	1.6000

AOB

Name	Default	Value
Yield [mgCOD/mgN]	0.1500	0.1500
AOB denite NO2 fraction as TEA [-]	0.5000	0.5000
Byproduct NH4 fraction to N2O [-]	0.0025	0.0025
N in biomass [mgN/mgCOD]	0.0700	0.0700
P in biomass [mgP/mgCOD]	0.0220	0.0220
Fraction to endogenous residue [-]	0.0800	0.0800
COD:VSS ratio [mgCOD/mgVSS]	1.4200	1.4200

NOB

Name	Default	Value
Yield [mgCOD/mgN]	0.0900	0.0900
N in biomass [mgN/mgCOD]	0.0700	0.0700
P in biomass [mgP/mgCOD]	0.0220	0.0220
Fraction to endogenous residue [-]	0.0800	0.0800
COD:VSS ratio [mgCOD/mgVSS]	1.4200	1.4200

AAO

Name	Default	Value
Yield [mgCOD/mgN]	0.1140	0.1140
Nitrate production [mgN/mgBiomassCOD]	2.2800	2.2800
N in biomass [mgN/mgCOD]	0.0700	0.0700
P in biomass [mgP/mgCOD]	0.0220	0.0220
Fraction to endogenous residue [-]	0.0800	0.0800
COD:VSS ratio [mgCOD/mgVSS]	1.4200	1.4200

OHO

Name	Default	Value
Yield (aerobic) [-]	0.6660	0.5000
Yield (fermentation, low H2) [-]	0.1000	0.1000
Yield (fermentation, high H2) [-]	0.1000	0.1000
H2 yield (fermentation low H2) [-]	0.3500	0.3500
H2 yield (fermentation high H2) [-]	0	0
Propionate yield (fermentation, low H2) [-]	0	0
Propionate yield (fermentation, high H2) [-]	0.7000	0.7000
CO2 yield (fermentation, low H2) [-]	0.7000	0.7000
CO2 yield (fermentation, high H2) [-]	0	0
N in biomass [mgN/mgCOD]	0.0700	0.0700
P in biomass [mgP/mgCOD]	0.0220	0.0220
Endogenous fraction - aerobic [-]	0.0800	0.0800
Endogenous fraction - anoxic [-]	0.1030	0.1030
Endogenous fraction - anaerobic [-]	0.1840	0.1840
COD:VSS ratio [mgCOD/mgVSS]	1.4200	1.4200
Yield (anoxic) [-]	0.5400	0.4500
Yield propionic (aerobic) [-]	0.6400	0.6400
Yield propionic (anoxic) [-]	0.4600	0.4600
Yield acetic (aerobic) [-]	0.6000	0.6000
Yield acetic (anoxic) [-]	0.4300	0.4300
Yield methanol (aerobic) [-]	0.5000	0.5000
Adsorp. max. [-]	1.0000	1.0000
Max fraction to N2O at high FNA over nitrate [-]	0.0500	0.0500
Max fraction to N2O at high FNA over nitrite [-]	0.1000	0.1000

PAO

Name	Default	Value
Yield (aerobic) [-]	0.6390	0.6390
Yield (anoxic) [-]	0.5200	0.5200
Aerobic P/PHA uptake [mgP/mgCOD]	0.9300	0.9300
Anoxic P/PHA uptake [mgP/mgCOD]	0.3500	0.3500
Yield of PHA on sequestration [-]	0.8890	0.8890
N in biomass [mgN/mgCOD]	0.0700	0.0700
N in sol. inert [mgN/mgCOD]	0.0700	0.0700
P in biomass [mgP/mgCOD]	0.0220	0.0220
Fraction to endogenous part. [-]	0.2500	0.2500
Inert fraction of endogenous sol. [-]	0.2000	0.2000
P/Ac release ratio [mgP/mgCOD]	0.5100	0.5100
COD:VSS ratio [mgCOD/mgVSS]	1.4200	1.4200
Yield of low PP [-]	0.9400	0.9400

Appendix B

Appendix 1- Steady state simulation of BNR process

Configuration information for thickener-fermenter for primary sludge

Physical data

Element name	Volume [m3]	Area [m2]	Depth [m]
Thickener	500.0000	125.0000	4.000
Fermenter	3600.0000	900.0000	4.000

Operating data Average (flow/time weighted as required)

Element name	Split method	Average Split specification
Thickener	Flowrate [Under]	60
Fermenter	Flowrate [Under]	1

Element name	Percent removal	Blanket fraction
Thickener	99.00	0.10
Fermenter	65.00	0.10

Configuration information for all Bioreactor units

Physical data

Element name	Volume [m3]	Area [m2]	Depth [m]	# of diffusers
Aerobic 1	4000.0000	888.8889	4.500	2168
Aerobic 3	4000.0000	888.8889	4.500	2168
Anaerobic	2000.0000	444.4444	4.500	Un-aerated
Anoxic 1	3000.0000	666.6667	4.500	Un-aerated
Anoxic 2	3000.0000	666.6667	4.500	Un-aerated
Aerobic 4	3500.0000	777.7778	4.500	1897
Aerobic 2	4000.0000	888.8889	4.500	2168

Operating data Average (flow/time weighted as required)

Element name	Average DO Setpoint [mg/L]
Aerobic 1	4.0
Aerobic 3	4.0
Anaerobic	0
Anoxic 1	0
Anoxic 2	0
Aerobic 4	2.0
Aerobic 2	4.0

Configuration information for Final Clarifier unit

Physical data

Element name	Volume[m3]	Area[m2]	Depth[m]	Number of layers	Top feed layer	Feed Layers
Secondary clarifier	6250.0000	1562.5000	4.000	10	6	1

Operating data Average (flow/time weighted as required)

Element name	Split method	Average Split specification
Secondary clarifier	Ratio	0.70

Element name	Average Temperature	Reactive
Secondary clarifier	Uses global setting	No

Local settling parameters

Element name	Maximum Vesilind settling velocity (Vo)	Vesilind hindered zone settling parameter (K) [L/g]	Clarification switching function [mg/L]	Specified TSS conc. for height calc. [mg/L]	Maximum compactability constant [mg/L]
Secondary clarifier	170.000	0.370	100.0000	2500.0000	1.500E+4

Influent information

Element name	Raw WW
Flow	60000
Total COD mgCOD/L	500.00
Total Kjeldahl Nitrogen mgN/L	45.00
Total P mgP/L	7.50
Nitrate N mgN/L	0
pH	7.90
Alkalinity mmol/L	7.00
ISS Influent mgISS/L	45.00
Calcium mg/L	80.00
Magnesium mg/L	15.00
Dissolved oxygen mg/L	0

Element name	Raw WW
Fbs - Readily biodegradable (including Acetate) [gCOD/g of total COD]	0.1600
Fac - Acetate [gCOD/g of readily biodegradable COD]	0.1500
Fxsp - Non-colloidal slowly biodegradable [gCOD/g of slowly degradable COD]	0.7500
Fus - Unbiodegradable soluble [gCOD/g of total COD]	0.0500
Fup - Unbiodegradable particulate [gCOD/g of total COD]	0.1300
Fna - Ammonia [gNH3-N/gTKN]	0.6600
Fnox - Particulate organic nitrogen [gN/g Organic N]	0.5000
Fnus - Soluble unbiodegradable TKN [gN/gTKN]	0.0200
FupN - N:COD ratio for unbiodegradable part. COD [gN/gCOD]	0.0350
Fpo4 - Phosphate [gPO4-P/gTP]	0.5000
FupP - P:COD ratio for unbiodegradable part. COD [gP/gCOD]	0.0110
FZbh - OHO COD fraction [gCOD/g of total COD]	0.0200
FZbm - Methylotroph COD fraction [gCOD/g of total COD]	1.000E-4
FZaob - AOB COD fraction [gCOD/g of total COD]	1.000E-4
FZnob - NOB COD fraction [gCOD/g of total COD]	1.000E-4
FZaao - AAO COD fraction [gCOD/g of total COD]	1.000E-4
FZbp - PAO COD fraction [gCOD/g of total COD]	1.000E-4
FZbpa - Propionic acetogens COD fraction [gCOD/g of total COD]	1.000E-4
FZbam - Acetoclastic methanogens COD fraction [gCOD/g of total COD]	1.000E-4
FZbhm - H2-utilizing methanogens COD fraction [gCOD/g of total COD]	1.000E-4
FZe - Endogenous products COD fraction [gCOD/g of total COD]	0

Configuration information Ideal primary settling tank

Physical data

Element name	Volume [m3]	Area [m2]	Depth [m]
Ideal primary settling tank9	5000	1250	4

Operating data Average (flow/time weighted as required)

Element name	Split method	Average Split specification
Primary Clarifier underflow	Flowrate [Under]	600

Element name	Percent removal	Blanket fraction
Primary Clarifier	65.00	0.10

Configuration information for all Splitter units

Operating data Average (flow/time weighted as required)

Element name	Split method	Average Split specification
WAS splitter	Flowrate [Side]	521 (SRT active control)
Internal recycle	Ratio	3.00
Influent distribution	Fraction	0.60
Valve to batch reactor	Routed	

Configuration information for P/COD addition stream

Operating data Average (flow/time weighted as required)

Element name	P/FCOD addition
Ordinary heterotrophic organisms (OHO) mgCOD/L	0
Methylotrophs mgCOD/L	0
Ammonia oxidizing biomass (AOB) mgCOD/L	0
Nitrite oxidizing biomass (NOB) mgCOD/L	0
Anaerobic ammonia oxidizers (AAO) mgCOD/L	0
Polyphosphate accumulating organisms (PAO) mgCOD/L	0
Propionic acetogens mgCOD/L	0
Methanogens - acetoclastic mgCOD/L	0
Methanogens - hydrogenotrophic mgCOD/L	0
Endogenous products mgCOD/L	0
Slowly bio. COD (part.) mgCOD/L	0
Slowly bio. COD (colloid.) mgCOD/L	0
Part. inert. COD mgCOD/L	0
Part. bio. org. N mgN/L	0
Part. bio. org. P mgP/L	0
Part. inert N mgN/L	0
Part. inert P mgP/L	0
Stored PHA mgCOD/L	0
Releasable stored polyP mgP/L	0
Fixed stored polyP mgP/L	0
Readily bio. COD (complex) mgCOD/L	59400 (FCOD addition)
Acetate mgCOD/L	0
Propionate mgCOD/L	0
Methanol mgCOD/L	0

Dissolved H2 mgCOD/L	0
Dissolved methane mg/L	0
Ammonia N mgN/L	0
Sol. bio. org. N mgN/L	0
Nitrous Oxide N mgN/L	0
Nitrite N mgN/L	0
Nitrate N mgN/L	0
Dissolved nitrogen gas mgN/L	0
PO4-P (Sol. & Me Complexed) mgP/L	50000.00 (P addition)
Sol. inert COD mgCOD/L	0
Sol. inert TKN mgN/L	0
ISS Influent mgISS/L	0
Struvite mgISS/L	0
Hydroxy-dicalcium-phosphate mgISS/L	0
Hydroxy-apatite mgISS/L	0
Magnesium mg/L	0
Calcium mg/L	0
Metal mg/L	0
Other Cations (strong bases) meq/L	0
Other Anions (strong acids) meq/L	0
Total CO2 mmol/L	0
User defined 1 mg/L	0
User defined 2 mg/L	0
User defined 3 mgVSS/L	0
User defined 4 mgISS/L	0
Dissolved oxygen mg/L	0
Flow	0.09999936 (P) or 10 (FCOD)

Kinetic and stoichiometric parameters and Mass transfer

Kinetic Parameters

Common

Name	Default	Value	Arrhenius
Hydrolysis rate [1/d]	2.1000	2.1000	1.0290
Hydrolysis half sat. [-]	0.0600	0.0600	1.0000
Anoxic hydrolysis factor [-]	0.2800	0.2800	1.0000
Anaerobic hydrolysis factor (AS) [-]	0.0400	0.0400	1.0000
Anaerobic hydrolysis factor (AD) [-]	0.5000	0.5000	1.0000
Adsorption rate of colloids [L/(mgCOD d)]	0.1500	0.1500	1.0290
Ammonification rate [L/(mgN d)]	0.0400	0.0400	1.0290
Assimilative nitrate/nitrite reduction rate [1/d]	0.5000	0.5000	1.0000
Endogenous products decay rate [1/d]	0	0	1.0000

AOB

Name	Default	Value	Arrhenius
Max. spec. growth rate [1/d]	0.9000	0.9000	1.0720
Substrate (NH4) half sat. [mgN/L]	0.7000	0.7000	1.0000
Byproduct NH4 logistic slope [-]	50.0000	50.0000	1.0000
Byproduct NH4 inflection point [mgN/L]	1.4000	1.4000	1.0000
AOB denite DO half sat. [mg/L]	0.1000	0.1000	1.0000
AOB denite HNO2 half sat. [mgN/L]	5.000E-6	5.000E-6	1.0000
Aerobic decay rate [1/d]	0.1700	0.1700	1.0290
Anoxic/anaerobic decay rate [1/d]	0.0800	0.0800	1.0290
KiHNO2 [mmol/L]	0.0050	0.0050	1.0000

NOB

Name	Default	Value	Arrhenius
Max. spec. growth rate [1/d]	0.7000	0.7000	1.0600
Substrate (NO2) half sat. [mgN/L]	0.1000	0.1000	1.0000
Aerobic decay rate [1/d]	0.1700	0.1700	1.0290
Anoxic/anaerobic decay rate [1/d]	0.0800	0.0800	1.0290
KiNH3 [mmol/L]	0.0750	0.0750	1.0000

AAO

Name	Default	Value	Arrhenius
Max. spec. growth rate [1/d]	0.2000	0.1000	1.1000
Substrate (NH4) half sat. [mgN/L]	2.0000	2.0000	1.0000
Substrate (NO2) half sat. [mgN/L]	1.0000	1.0000	1.0000
Aerobic decay rate [1/d]	0.0190	0.0190	1.0290
Anoxic/anaerobic decay rate [1/d]	0.0095	0.0095	1.0290
Ki Nitrite [mgN/L]	1000.0000	1000.0000	1.0000
Nitrite sensitivity constant [L / (d mgN)]	0.0160	0.0160	1.0000

OHO

Name	Default	Value	Arrhenius
Max. spec. growth rate [1/d]	3.2000	3.2000	1.0290
Substrate half sat. [mgCOD/L]	5.0000	5.0000	1.0000
Anoxic growth factor [-]	0.5000	0.5000	1.0000
Denite N2 producers (NO3 or NO2) [-]	0.5000	0.5000	1.0000
Aerobic decay rate [1/d]	0.6200	0.6200	1.0290
Anoxic decay rate [1/d]	0.2330	0.2330	1.0290
Anaerobic decay rate [1/d]	0.1310	0.1310	1.0290
Fermentation rate [1/d]	1.6000	1.6000	1.0290
Fermentation half sat. [mgCOD/L]	5.0000	5.0000	1.0000
Fermentation growth factor (AS) [-]	0.2500	0.2500	1.0000
Free nitrous acid inhibition [mmol/L]	1.000E-7	1.000E-7	1.0000

PAO

Name	Default	Value	Arrhenius
Max. spec. growth rate [1/d]	0.9500	0.9500	1.0000
Max. spec. growth rate, P-limited [1/d]	0.4200	0.4200	1.0000
Substrate half sat. [mgCOD(PHB)/mgCOD(Zbp)]	0.1000	0.1000	1.0000
Substrate half sat., P-limited [mgCOD(PHB)/mgCOD(Zbp)]	0.0500	0.0500	1.0000
Magnesium half sat. [mgMg/L]	0.1000	0.1000	1.0000
Cation half sat. [mmol/L]	0.1000	0.1000	1.0000
Calcium half sat. [mgCa/L]	0.1000	0.1000	1.0000
Aerobic/anoxic decay rate [1/d]	0.1000	0.1000	1.0000
Aerobic/anoxic maintenance rate [1/d]	0	0	1.0000
Anaerobic decay rate [1/d]	0.0400	0.0400	1.0000
Anaerobic maintenance rate [1/d]	0	0	1.0000
Sequestration rate [1/d]	4.5000	4.5000	1.0000
Anoxic growth factor [-]	0.3300	0.3300	1.0000

pH

Name	Default	Value
OHO low pH limit [-]	4.0000	4.0000
OHO high pH limit [-]	10.0000	10.0000
Methylotrophs low pH limit [-]	4.0000	4.0000
Methylotrophs high pH limit [-]	10.0000	10.0000
Autotrophs low pH limit [-]	5.5000	5.5000
Autotrophs high pH limit [-]	9.5000	9.5000
PAO low pH limit [-]	4.0000	4.0000
PAO high pH limit [-]	10.0000	10.0000
OHO low pH limit (anaerobic) [-]	5.5000	5.5000
OHO high pH limit (anaerobic) [-]	8.5000	8.5000
Propionic acetogens low pH limit [-]	4.0000	4.0000
Propionic acetogens high pH limit [-]	10.0000	10.0000
Acetoclastic methanogens low pH limit [-]	5.0000	5.0000
Acetoclastic methanogens high pH limit [-]	9.0000	9.0000
H2-utilizing methanogens low pH limit [-]	5.0000	5.0000
H2-utilizing methanogens high pH limit [-]	9.0000	9.0000

Switches

Name	Default	Value
Aerobic/anoxic DO half sat. [mgO2/L]	0.0500	0.0500
Anoxic/anaerobic NOx half sat. [mgN/L]	0.1500	0.1500
AOB DO half sat. [mgO2/L]	0.2500	0.2500
NOB DO half sat. [mgO2/L]	0.5000	0.5000
AAO DO half sat. [mgO2/L]	0.0100	0.0100
Anoxic NO3(->NO2) half sat. [mgN/L]	0.1000	0.1000
Anoxic NO3(->N2) half sat. [mgN/L]	0.0500	0.0500
Anoxic NO2(->N2) half sat. (mgN/L)	0.0100	0.0100
NH3 nutrient half sat. [mgN/L]	0.0050	0.0050
PolyP half sat. [mgP/mgCOD]	0.0100	0.0100
VFA sequestration half sat. [mgCOD/L]	5.0000	5.0000
P uptake half sat. [mgP/L]	0.1500	0.1500
P nutrient half sat. [mgP/L]	0.0010	0.0010
Autotroph CO2 half sat. [mmol/L]	0.1000	0.1000
H2 low/high half sat. [mgCOD/L]	1.0000	1.0000
Propionic acetogens H2 inhibition [mgCOD/L]	5.0000	5.0000
Synthesis anion/cation half sat. [meq/L]	0.0100	0.0100

Stoichiometric Parameters

Common

Name	Default	Value
Biomass volatile fraction (VSS/TSS)	0.9200	0.9200
Endogenous residue volatile fraction (VSS/TSS)	0.9200	0.9200
N in endogenous residue [mgN/mgCOD]	0.0700	0.0700
P in endogenous residue [mgP/mgCOD]	0.0220	0.0220
Endogenous residue COD:VSS ratio [mgCOD/mgVSS]	1.4200	1.4200
Particulate substrate COD:VSS ratio [mgCOD/mgVSS]	1.6000	1.6000
Particulate inert COD:VSS ratio [mgCOD/mgVSS]	1.6000	1.6000

AOB

Name	Default	Value
Yield [mgCOD/mgN]	0.1500	0.1500
AOB denite NO2 fraction as TEA [-]	0.5000	0.5000
Byproduct NH4 fraction to N2O [-]	0.0025	0.0025
N in biomass [mgN/mgCOD]	0.0700	0.0700
P in biomass [mgP/mgCOD]	0.0220	0.0220
Fraction to endogenous residue [-]	0.0800	0.0800
COD:VSS ratio [mgCOD/mgVSS]	1.4200	1.4200

NOB

Name	Default	Value
Yield [mgCOD/mgN]	0.0900	0.0900
N in biomass [mgN/mgCOD]	0.0700	0.0700
P in biomass [mgP/mgCOD]	0.0220	0.0220
Fraction to endogenous residue [-]	0.0800	0.0800
COD:VSS ratio [mgCOD/mgVSS]	1.4200	1.4200

AAO

Name	Default	Value
Yield [mgCOD/mgN]	0.1140	0.1140
Nitrate production [mgN/mgBiomassCOD]	2.2800	2.2800
N in biomass [mgN/mgCOD]	0.0700	0.0700
P in biomass [mgP/mgCOD]	0.0220	0.0220
Fraction to endogenous residue [-]	0.0800	0.0800
COD:VSS ratio [mgCOD/mgVSS]	1.4200	1.4200

OHO

Name	Default	Value
Yield (aerobic) [-]	0.6660	0.6660
Yield (fermentation, low H2) [-]	0.1000	0.1000
Yield (fermentation, high H2) [-]	0.1000	0.1000
H2 yield (fermentation low H2) [-]	0.3500	0.3500
H2 yield (fermentation high H2) [-]	0	0
Propionate yield (fermentation, low H2) [-]	0	0
Propionate yield (fermentation, high H2) [-]	0.7000	0.7000
CO2 yield (fermentation, low H2) [-]	0.7000	0.7000
CO2 yield (fermentation, high H2) [-]	0	0
N in biomass [mgN/mgCOD]	0.0700	0.0700
P in biomass [mgP/mgCOD]	0.0220	0.0220
Endogenous fraction - aerobic [-]	0.0800	0.0800
Endogenous fraction - anoxic [-]	0.1030	0.1030
Endogenous fraction - anaerobic [-]	0.1840	0.1840
COD:VSS ratio [mgCOD/mgVSS]	1.4200	1.4200
Yield (anoxic) [-]	0.5400	0.5400
Yield propionic (aerobic) [-]	0.6400	0.6400
Yield propionic (anoxic) [-]	0.4600	0.4600
Yield acetic (aerobic) [-]	0.6000	0.6000
Yield acetic (anoxic) [-]	0.4300	0.4300
Yield methanol (aerobic) [-]	0.5000	0.5000
Adsorp. max. [-]	1.0000	1.0000
Max fraction to N2O at high FNA over nitrate [-]	0.0500	0.0500
Max fraction to N2O at high FNA over nitrite [-]	0.1000	0.1000

PAO

Name	Default	Value
Yield (aerobic) [-]	0.6390	0.6390
Yield (anoxic) [-]	0.5200	0.5200
Aerobic P/PHA uptake [mgP/mgCOD]	0.9300	0.9300
Anoxic P/PHA uptake [mgP/mgCOD]	0.3500	0.3500
Yield of PHA on sequestration [-]	0.8890	0.8890
N in biomass [mgN/mgCOD]	0.0700	0.0700
N in sol. inert [mgN/mgCOD]	0.0700	0.0700
P in biomass [mgP/mgCOD]	0.0220	0.0220
Fraction to endogenous part. [-]	0.2500	0.2500
Inert fraction of endogenous sol. [-]	0.2000	0.2000
P/Ac release ratio [mgP/mgCOD]	0.5100	0.5100
COD:VSS ratio [mgCOD/mgVSS]	1.4200	1.4200
Yield of low PP [-]	0.9400	0.9400

Mass transfer

Name	Default	Value	Arrhenius
Kl for H2 [m/d]	17.0000	17.0000	1.0240
Kl for CO2 [m/d]	10.0000	10.0000	1.0240
Kl for NH3 [m/d]	1.0000	1.0000	1.0240
Kl for CH4 [m/d]	8.0000	8.0000	1.0240
Kl for N2 [m/d]	15.0000	15.0000	1.0240
Kl for N2O [m/d]	8.0000	8.0000	1.0240
Kl for O2 [m/d]	13.0000	13.0000	1.0240

Appendix C

Dynamic simulation of anaerobic batch test

The anaerobic batch tests of BNR sludge was simulated using the unit "variable volume reactor" of the simulator (named batch reactor in **Error! Reference source not found.**). The batch reactor was in line with the process for 1 d to direct the RAS to the batch reactor. Then, the batch reactor was by-passed in process. The batch reactor was simulated (dynamic) for on cycle of anaerobic –aerobic conditions. For anaerobic stage, DO was set 0 mg L-1 and HRT of anaerobic condition was changed to 0, 12, 24 and 48 at four different dynamic simulations. In each simulation after anaerobic stage, DO was set 4 mg L^{-1} for 48 h to measure the aerobic phosphorous uptake was of sludge.

The modelling of activated sludge under hydrolysis/fermentation conditions with regard to its phosphorous removal performance has not been yet investigated. The only study by Barnard et al. (2015) used BioWin simulation to predict the effect of mixed liquor fermentation on phosphorous removal performance of a pilot-scale BNR process (5-stage Bardenpho). In that study, the VFA production in fermenter was calibrated with the rate of anaerobic hydrolysis process (the FCOD production in the model was fit to the results of batch test fermentation of activated sludge). This method was based on the assumption that all FCOD produced through hydrolysis was fermented and could be used by PAO. Therefore when poly-P is available in sludge, FCOD would not accumulate in the system. This prediction is not consistent with the results of experimental studies. In this study in the test with BNR sludge, during period of h 0 - 12, 15.3 mg COD gVSS-1 of FCOD was accumulated in the system; while, sludge still had 13 mg PO_4^{-3}-P $gVSS^{-1}$ of poly-P at h 12 (Figure 2b and d). At meantime, 16 mg $PO4^{-3}$-P $gVSS^{-1}$ of

poly-P was utilized to uptake about 37 mg COD gVSS-1 of VFA (estimated using the anaerobic phosphorous release to COD uptake ratio of 0.43 mg PO_4^{-3}-P mg^{-1} COD (SATOH et al., 1996; Wentzel et al., 1986). Thus the ratio of VFA to total FCOD produced (sum of non-VFA FCOD accumulated and VFA-FCOD stored) was 0.71. In the batch fermentation test (SRT=5 days) conducted on activated sludge from different full-scale treatment plants, the ratio of VFA to FCOD accumulated (VFA/FCOD) ranged from 22 to 92% (Ucisik and Henze, 2008). Vollertsen et al. (2006) found that about half of FCOD produced in SHT (HRT= 1.5 d) could not be used by PAO. The FCOD that accumulates in SHT returns back to mainstream BNR process and could be used for denitrification. This could affect the overall effect of SHT on BNR process, thus needs to be accounted. To do so in this study, both the FCOD production and poly-P utilization (equal to VFA production) were calibrated by adjusting the rate of anaerobic hydrolysis and fermentation processes (details of calibration are presented below).

Configuration information for all Variable volume bioreactor units

Physical data

Element name	Volume [m3]	Area [m2]	Depth [m]	# of diffusers
Batch Reactor	500.0000	111.1111	4.500	271

Operating data Average (flow/time weighted as required)

Element name	Average DO Setpoint [mg/L]
Bacth Reactor	0.8

Element name	Average Temperature [deg. C]

Bacth Reactor	22.0

Local biological parameters

Element name	Max. spec. growth rate [1/d]	Substrate (NH4) half sat. [mgN/L]	Byproduct NH4 logistic slope [-]	Byproduct NH4 inflection point [mgN/L]	AOB denite DO half sat. [mg/L]	AOB denite HNO2 half sat. [mgN/L]	Aerobic decay rate [1/d]	Anoxic/anaerobic decay rate [1/d]	KiHNO2 [mmol/L]
Bacth Reactor	0.9000	0.7000	50.0000	1.4000	0.1000	5.000E-6	0.1700	0.0800	0.0050

element name	Max. spec. growth rate [1/d]	Substrate (NO2) half sat. [mgN/L]	Aerobic decay rate [1/d]	Anoxic/anaerobic decay rate [1/d]	KiNH3 [mmol/L]
Bacth Reactor	0.7000	0.1000	0.1700	0.0800	0.0750

Element name	Max. spec. growth rate [1/d]	Substrate (NH4) half sat. [mgN/L]	Substrate (NO2) half sat. [mgN/L]	Aerobic decay rate [1/d]	Anoxic/anaerobic decay rate [1/d]	Ki Nitrite [mgN/L]	Nitrite sensitivity constant [L / (d mgN)]
Bacth Reactor	0.1000	2.0000	1.0000	0.0190	0.0095	1000.0000	0.0160

Element name	Hydrolysis rate [1/d]	Hydrolysis half sat. [-]	Anoxic hydrolysis factor [-]	Anaerobic hydrolysis factor (AS) [-]	Anaerobic hydrolysis factor (AD) [-]	Adsorption rate of colloids [L/(mgCOD d)]	Ammonification rate [L/(mgN d)]	Assimilative nitrate/nitrite reduction rate [1/d]	Endogenous products decay rate [1/d]
Bacth Reactor	2.1000	0.0600	0.2800	0.2000	0.5000	0.1500	0.0400	0.5000	0

Element name	Bacth Reactor
Max. spec. growth rate [1/d]	0.95
Max. spec. growth rate, P-limited [1/d]	0.42
Substrate half sat. [mgCOD(PHB)/mgCOD(Zbp)]	0.1
Substrate half sat., P-limited [mgCOD(PHB)/mgCOD(Zbp)]	0.05
Magnesium half sat. [mgMg/L]	0.1
Cation half sat. [mmol/L]	0.1
Calcium half sat. [mgCa/L]	0.1
Aerobic/anoxic decay rate [1/d]	0.1
Aerobic/anoxic maintenance rate [1/d]	0
Anaerobic decay rate [1/d]	0.04
Anaerobic maintenance rate [1/d]	0
Sequestration rate [1/d]	4.5
Anoxic growth factor [-]	0.33

Element name	Max. spec. growth rate [1/d]	Methanol half sat. [mgCOD/L]	Denite N2 producers (NO3 or NO2) [-]	Aerobic decay rate [1/d]	Anoxic/anaerobic decay rate [1/d]	Free nitrous acid inhibition [mmol/L]
Bacth Reactor	1.3000	0.5000	0.5000	0.0400	0.0300	1.000E-7

Element name	Max. spec. growth rate [1/d]	Substrate half sat. [mgCOD/L]	Acetate inhibition [mgCOD/L]	Anaerobic decay rate [1/d]	Aerobic/anoxic decay rate [1/d]
Bacth Reactor	0.2500	10.0000	10000.0000	0.0500	0.5200

Element name	Bacth Reactor
Acetoclastic max. spec. growth rate [1/d]	0.3
H2-utilizing max. spec. growth rate [1/d]	1.4
Acetoclastic substrate half sat. [mgCOD/L]	100
Acetoclastic methanol half sat. [mgCOD/L]	0.5
H2-utilizing CO2 half sat. [mmol/L]	0.1
H2-utilizing substrate half sat. [mgCOD/L]	0.1
H2-utilizing methanol half sat. [mgCOD/L]	0.5
Acetoclastic propionic inhibition [mgCOD/L]	10000
Acetoclastic anaerobic decay rate [1/d]	0.13
Acetoclastic aerobic/anoxic decay rate [1/d]	0.6
H2-utilizing anaerobic decay rate [1/d]	0.13
H2-utilizing aerobic/anoxic decay rate [1/d]	2.8

Element name	Bacth Reactor
OHO low pH limit [-]	4
OHO high pH limit [-]	10
Methylotrophs low pH limit [-]	4
Methylotrophs high pH limit [-]	10
Autotrophs low pH limit [-]	5.5
Autotrophs high pH limit [-]	9.5
PAO low pH limit [-]	4
PAO high pH limit [-]	10
OHO low pH limit (anaerobic) [-]	5.5
OHO high pH limit (anaerobic) [-]	8.5
Propionic acetogens low pH limit [-]	4
Propionic acetogens high pH limit [-]	10
Acetoclastic methanogens low pH limit [-]	5
Acetoclastic methanogens high pH limit [-]	9
H2-utilizing methanogens low pH limit [-]	5
H2-utilizing methanogens high pH limit [-]	9

Element name	Bacth Reactor
Aerobic/anoxic DO half sat. [mgO2/L]	0.05
Anoxic/anaerobic NOx half sat. [mgN/L]	0.15
AOB DO half sat. [mgO2/L]	0.25
NOB DO half sat. [mgO2/L]	0.5
AAO DO half sat. [mgO2/L]	0.01
Anoxic NO3(->NO2) half sat. [mgN/L]	0.1
Anoxic NO3(->N2) half sat. [mgN/L]	0.05
Anoxic NO2(->N2) half sat. (mgN/L)	0.01
NH3 nutrient half sat. [mgN/L]	0.005
PolyP half sat. [mgP/mgCOD]	0.01
VFA sequestration half sat. [mgCOD/L]	5
P uptake half sat. [mgP/L]	0.15
P nutrient half sat. [mgP/L]	0.001
Autotroph CO2 half sat. [mmol/L]	0.1
H2 low/high half sat. [mgCOD/L]	1
Propionic acetogens H2 inhibition [mgCOD/L]	5
Synthesis anion/cation half sat. [meq/L]	0.01

Aeration equipment parameters

Element name	k1 in C = k1(PC)^0.25 + k2	k2 in C = k1(PC)^0.25 + k2	Y in Kla = C Usg ^ Y - Usg in [m3/(m2 d)]	Area of one diffuser	% of tank area covered by diffusers [%]
Bacth Reactor	2.5656	0.0432	0.8200	0.0410	10.0000

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